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by

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Genetic Homeostasis is devoted to the discussion of the self-regulating properties of Mendelian populations. The book is primarily intended for students of evolution, geneticists and workers in animal and plant improvement. Following a brief introduction, it is divided into two parts. The first considers evidence from a variety of sources on different species, including previously unpublished data from the author's laboratory. The second discusses and interprets the evidence from the standpoints of evolution and of application to breeding practice. The concept that interbreeding groups of organisms in nature or under domestication possess certain characteristics, which transcend those of their individual members, is elaborated. A hypothesis seeking to explain the nature of such properties is advanced. The basic feature of the theory proposed is that natural selection favours individuals which are well canalized, that is to say, organisms which possess auto-regulating or buffering powers to stay within normal channels of development. If such cybernetic control depends on a hybrid or heterozygous genetic constitution, populations as a whole become endowed with phenotypic uniformity without losing their genetic diversity, both being evolutionary properties necessary for their successful existence. *Demy 8vo.* 144 *pages.* **12/6** *net.*

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ON TETRAD ANALYSES APPARENTLY INCONSISTENT WITH MENDELIAN LAW

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I. INTRODUCTION

At the Seventh International Botanical Congress in Stockholm in 1950, Professor I. E. Glushchenko (Russia) presented a paper which gave rise to a lively discussion of mendelian segregation, whose universal validity and dependence upon a chromosome mechanism was not admitted by the Russian investigator. On this occasion Professor A. Quintanilha (Portugal) asked Glushchenko how he would explain, other than by a chromosome mechanism, the simple 2 : 2 segregations which result from tetrad analyses of various monohybrid Cryptogams such as *Chlamydomonas*, *Coprinus*, *Neurospora* and *Saccharomyces*. Glushchenko replied that he was not familiar with these organisms but assumed that the results were due to a statistical phenomenon and that other numerical ratios besides the 2 : 2 should occur. To this statement Quintanilha replied that it was not a question of statistics, but rather of a fundamental principle, according to which every ascus or basidium of a monohybrid yields a segregation in the ratio of 2 : 2.

As is well known, Hans Winkler in 1930 put forward his conversion theory, according to which a gene in a heterozygote may be converted into the allemorphic gene, so that a dominant gene may be changed into a recessive and vice versa. Also, according to this theory, a whole group of linked genes may be transformed at one step into a corresponding group of allelomorphs. Furthermore, the frequency of conversion of a dominant gene in the recessive direction need not always be the same as that of a recessive gene in the dominant direction. Winkler's principal aim was to show that T. H. Morgan's theory of crossing over need not be correct, since gene conversion could also explain those phenomena normally explainable on the basis of crossing over. Winkler did not, however, deny the position of genes along the length of the chromosome.

An important part of Winkler's argument was the fact that several investigators had found that tetrad analyses of certain Cryptogams sometimes yielded segregation ratios deviating from the expected 2 : 2 (Fr. Wettstein in *Funaria hygrometrica*, Brunswick in *Coprinus fimetarius*, Kniep in *Aleurodiscus polygonius*, Hanna in *Ustilago zeae*, etc.)—exceptions which could be accounted for on the basis of gene conversion.

In general, Winkler's theory received little support until Lindegren and his associates (Lindegren, 1949, 1953 ; Mundkur, 1949), on the basis of frequent deviations from the expected 2 : 2 ratios in the

tetrad analyses of yeasts (*Saccharomyces*), subscribed to it with much enthusiasm. They found quite commonly 3 dominant : 1 recessive segregations or 4 d : 0 r, 1 d : 3 r, and 0 d : 4 r and were thereby of the opinion that they had given the death blow to mendelism, or, at least, that mendelian theory needed a profound modification.

In what follows we will disregard the numerous sources of error which should be considered in tetrad analyses : (1) *overlapping of generations*, which carries with it a grave risk in the study of yeast genetics, (2) *mutation*, which here just as in all other organisms can lead to deviations from the expected segregation, (3) *adaptation* to the ability to ferment certain sugars or to grow in the absence of certain growth substances, which can lead to analytical difficulties, (4) *cytoplasmic inheritance* of certain respiratory enzymes, which has been shown through the work of Ephrussi (1953) and his associates to exist in yeasts, and (5) *polyploidy*, which could easily lead to abnormal segregation, although its occurrence in yeasts has not yet been established with certainty.

After not having found during the course of several years in our yeast genetic investigations any deviations from mendelian segregation which could not be explained on the basis of mutation, we observed (Winge and Roberts, 1950*a*) in a monohybrid heterozygous for maltose fermentation, *Mm*, a single 4-spored ascus (among 13) which yielded a 3 *M* : 1 *m* segregation. As a working hypothesis, we assumed that an extra mitosis had taken place in the young 4-nucleate ascus so that 4 *M*- and 4 *m*-nuclei were produced, but that only 4 uninucleate spores were formed and that these, by chance, contained 3 *M*-nuclei and 1 *m*-nucleus while the fourth *M*-nucleus and the 3 remaining *m*-nuclei were not incorporated in any spore. In support of this hypothesis we stated that both 5- and 6-spored asci had been observed, showing that the occurrence of extra mitoses was actually demonstrable in this material. Genetic analyses of these asci with more than 4 spores showed that in a single ascus either maltose fermenters or non-fermenters could be in excess.

This explanation was rejected by Mundkur (1950) who showed that the deviating segregation ratios in his own material, which originated from Lindegren and which contained a series of genes in the heterozygous condition, were not explainable on the basis of our hypothesis. He correctly argued that even if an ascus becomes 8-nucleate it could never contain more than 4 biotypes, and that to adopt this hypothesis for explaining his results would require the further assumption that more than 4 biotypes would have to be present among the assumed 8 nuclei in the ascus. It should be noted that we had never maintained that our hypothesis could explain all exceptions to the expected 2 : 2 ratios in tetrad analyses. As it will appear from the following, however, our hypothesis of the significance of extra mitoses actually contained the correct idea but lacked an important corrective.

2. GENETIC ANALYSIS OF DEVIATING ASCI IN OUR MATERIAL

In material originating from a hybridisation between *Saccharomyces cerevisiae*, type Yeast Foam and *S. chevalieri* we obtained, following various spore crossings, a hybrid (230) which was heterozygous in two gene pairs, $M_3m_3R_2r_2$—*i.e.* it was heterozygous with respect to one of the four maltose fermentation genes previously isolated and to one of three raffinose fermentation genes previously isolated (Winge and Roberts, 1948, 1950*b*, 1952; Gilliland, 1949). This hybrid yielded out of a total of 37 tetrads, 6 which gave ratios other than

TABLE 1

Abnormally segregating asci from hybrids 230, 254 and 236 all Mm Rr

Hybrid	No. of asci	$M+R$	$M+r$	$m+R$	$m+r$	
230	1	...	2	1	1	= 1 R : 3 r
	1	1	1	2	...	= 3 R : 1 r
	2	2	...	1	1	= 3 R : 1 r
	1	2	...	2	...	= 4 R : 0 r
	1	1	2	1	...	= 3 M : 1 m
254	1	1	1	...	2	= 1 R : 3 r
	3	2	1	1	...	= 3 M : 1 m / 3 R : 1 r
236	1	2	2	...	...	= 4 M : 0 m
	1	1	1	2	...	= 3 R : 1 r

2 : 2; these comprised 3 M : 1 m (1 ascus), 3 R : 1 r (3 asci), 1 R : 3 r (1 ascus), and 4 R : 0 r (1 ascus). The last-named ascus was undoubtedly the result of overlapping of generations.

Another hybrid (254), having the same origin and genetic formula as H 230, $M_3m_3R_2r_2$, yielded deviating ratios also. We analysed 107 tetrads from H 254, of which 103 segregated out normally (2 M : 2 m and 2 R : 2 r), while the four irregular asci comprised 3 cases of 3 M : 1 m+3 R : 1 r and one 1 R : 3 r.

Finally, a third doubly heterozygous hybrid (236) was produced through spore crossing. It originated from H 230 and had exactly the same formula, $M_3m_3R_2r_2$. In addition to 17 normally segregating asci (2 M : 2 m+2 R : 2 r) there were two deviating tetrads, one which gave a 4 M : 0 m+2 R : 2 r segregation, and one which gave a 3 R : 1 r+2 M : 2 m segregation.

We thus had ample material to investigate in the hope of explaining the occurrence of these abnormally segregating asci, which are shown in table 1.

These 12 deviating asci constituted 7·4 per cent. of the total number of asci analysed, of which 151 segregated normally. In

order to determine the cause of these deviations we undertook both genetical and cytological investigations of the material. A single case will serve to illustrate the results of the genetic investigations.

In table 1 the second deviating ascus of H 230 contained 4 spores (Nos. 25, 26, 27 and 28) which gave rise to 4 single-spore cultures which, on the basis of their fermentative ability, had the genetic formulas

$$M+R \quad M+r \quad m+R \text{ and } m+R$$

and consequently this tetrad segregated out as 2 M : 2 $m+3$ R : 1 r.

Since all of our yeast types contain the gene D for self-diploidisation (Winge and Roberts, 1949), it was to be expected that all spores isolated would give rise to homozygous single-spore cultures. We obtained, however, by isolating spores from each of the four diploid single-spore cultures of this deviating tetrad and analysing the subsequent cultures the following segregation ratios with respect to maltose and raffinose fermentative ability :

	Single-spore culture 230-25 (*M*+*r*)			Single-spore culture 230-26 (*M*+*R*)			Single-spore culture 230-27 (*m*+*R*)			Single-spore culture 230-28 (*m*+*R*)		
	5 asci			8 asci			2 asci			4 asci		
	4 : 0	2 : 2	0 : 4	4 : 0	2 : 2	0 : 4	4 : 0	2 : 2	0 : 4	4 : 0	2 : 2	0 : 4
Maltose .	5	0	0	0	8	0	0	0	2	0	0	4
Raffinose .	0	0	5	0	8	0	2	0	0	4	0	0
	Homozygous *MMrr*			Heterozygous *MmRr*			Homozygous *mmRR*			Homozygous *mmRR*		

It is seen that three of the four single-spore cultures were homozygous, *MMrr*, *mmRR*, and *mmRR*, while the fourth (230-26) was heterozygous in both gene pairs, *MmRr*.

Since this deviating ascus, 230-25 to 28, undoubtedly should have given a dicratic segregation of the type 2 *Mr* : 2 *mR*, it is evident that it is one of the *Mr*-spores which was abnormal, in that this spore gave rise to a culture able to ferment both maltose and raffinose. It can be concluded that an extra *mR*-nucleus was incorporated in the spore together with the theoretically expected *Mr*-nucleus, and this is not difficult to explain : *an extra mitosis occurred in the ascus, and the abnormal spore was binucleate, receiving an* mR-*nucleus in addition to its original* Mr-*nucleus ; Single-spore Culture 230-26 thus became doubly heterozygous.*

It is worthy of note in this connection that, as a matter of principle, if an ascus yields a dicratic segregation—*i.e.* either 2 *MR* : 2 *mr* or 2 *Mr* : 2 *mR* and a second nucleus arising from an extra mitosis is incorporated in a spore, then two possibilities may occur : (1) the extra nucleus is of the same genetic constitution as the original nucleus whereby the binucleate condition of the spore could not be detected, or (2) the extra nucleus is genetically different from the original spore nucleus, whereby the subsequent diploid culture is necessarily *doubly* heterozygous—either *MR*+*mr* or *Mr*+*mR*. In tetracratic asci

(*MR* : *Mr* : *mR* : *mr*), however, singly heterozygous single-spore cultures may arise from binucleate spores.

We investigated, in the same way as described above, the single-spore cultures of 6 of the 12 deviating asci listed in table 1. In 4 of these asci it was found that one of the four single-spore cultures was doubly heterozygous. In the fifth ascus one of the four single-spore cultures was singly heterozygous (*MmRR*), and in agreement with the above, this ascus gave a tetracratic segregation, the three spores which gave rise to the homozygous cultures having the formulas *MR*, *Mr*, and *mR*. The sixth and final ascus we investigated yielded 4 homozygous cultures, *MMRR*, *MMrr*, *mmRR*, and *mmRR*, but it is obvious that also this case should theoretically be expected to occur. If such a deviating tetracratic ascus becomes 8-nucleate and 4-spored, the subsequent diploid single-spore cultures can naturally have arisen through diploidisation of the 4 haploid spores, *MR*, *Mr*, *mR*, and *mR*. Here, the two *mr*-nuclei are not incorporated in any of the four spores.

Additional investigations, which will not be discussed in detail here, have shown that recessive *mr-spores* germinate more poorly than *MR*-, *Mr*-, and *mR*-spores, and it is possible that *mr-nuclei* cannot satisfactorily compete with the other nuclear types for the available cytoplasm. Analyses of tetrads in which all 4 spores germinate have disclosed the fact that *mr*-spores germinate poorly, for the dicratic ascus type 2 *MR* : 2 *mr* often occurred with a lower frequency than the dicratic ascus type 2 *Mr* : 2 *mR*, even though these two types, in the absence of linkage, should occur with equal frequencies.

3. GENETIC INVESTIGATIONS OF LINDEGREN'S HAPLOID YEASTS

Professor Carl Lindegren, Southern Illinois University, Carbondale, Illinois, kindly supplied us with two haploid cultures from his collection, one of which could ferment maltose while the other could not. These haploid cultures were of opposite mating types and were designated as

1428—α *ma*

15517—*a MA*

since Lindegren does not, as we, designate the allelomorphs for maltose fermentation with *M* and *m*, but with *MA* and *ma*.

Since it is primarily Lindegren who has obtained so many tetrads giving deviating segregation ratios, which he would explain on the basis of gene conversion, this material was of special interest. It is worthy of note in this connection that Lindegren's two cultures lack the *D* gene and therefore ascospores isolated from them normally give rise to haploid single-spore cultures. It was therefore a question whether our explanation of the deviating tetrads involving extra mitoses and binucleate spores was also valid for Lindegren's material.

The question can be definitely answered in the affirmative.

We produced a hybrid mixture between cultures 1428 and 15517 having the formula αa *MAma*, and we examined the segregation

ratios of 34 asci with the result that 31 segregated normally, 2 *MA* : 2 *ma*, while 3 asci gave a 3 *MA* : 1 *ma* segregation. We could thus confirm Lindegren's observations of irregular segregation in his material, but the explanation of this phenomenon has nothing in common with gene conversion, for in all 3 deviating asci we found that one of the three dominant *MA* types was diploid and heterozygous while the three remaining cultures in the tetrad were haploid. These three irregular tetrads had the following formulas :

MAma *MA* *MA* *ma*

As expected, the three haploid cultures were unable to sporulate, while the fourth culture produced spores.

Three asci from the diploid culture of the first irregular tetrad were analysed, and 2 segregated out as 2 *MA* : 2 *ma* and 1 as 1 *MA* : 3 *ma*.

Five asci from the diploid culture of the second irregular tetrad were analysed, and 4 segregated out as 2 *MA* : 2 *ma* and 1 as 3 *MA* : 1 *ma*.

Two asci from the diploid culture of the third irregular tetrad were analysed, and both segregated out as 2 *MA* : 2 *ma*.

Thus two out of the ten asci we analysed showed again abnormal segregation, and there is no doubt that Lindegren's material, just as ours in the present investigation, possesses an inherited tendency toward extra mitoses in the ascus as well as toward the formation of binucleate spores.

In this connection the researches of Mundkur (1950) are of interest, since he also worked with Lindegren's material. As previously mentioned, Mundkur was correct in denying the possibility that our explanation of irregular segregation involving supernumerary mitoses could be employed for his results. It will be seen, however, that the fact that spores may sometimes be binucleate can account for irregular segregations, which Mundkur (as well as Lindegren) would explain on the basis of gene conversion.

Let us take as an example the tetrad mentioned by Mundkur in 1950 which yielded abnormal segregation ratios. The spores of this tetrad had the following formulas, in spite of the fact that the hybrid from which they arose was heterozygous with respect to all genes investigated :

M 532	α	*G*	*ME*	*MG*	*MA*	*pn*	*in*
M 533	α	*G*	*ME*	*MG*	*ma*	*pn*	*in*
M 534	*a*	*g*	*ME*	*MG*	*MA*	*PN*	*IN*
M 535	*a*	*g*	*me*	*MG*	*MA*	*PN*	*IN*

(We have purposely omitted the gene pair *ad* (*P*)—*AD* (*W*) because it behaves in a special way ; the adenine-requiring red type, *ad* (*P*), very often " mutates " to a white phenotype, *ad* (*W*), which is methionine-dependent.)

It can be seen that from this tetrad there have been segregated out 3 *ME* : 1 *me*, 4 *MG* : 0 *mg*, and 3 *MA* : 1 *ma*. These deviating ratios can be reasonably explained in the following way.

Originally, the 4 primary nuclei in the ascus were :

1 :	α	*G*	*ME*	*MG*	*MA*	*pn*	*in*
2 :	α	*G*	*me*	*mg*	*ma*	*pn*	*in*
3 :	*a*	*g*	*ME*	*mg*	*MA*	*PN*	*IN*
4 :	*a*	*g*	*me*	*MG*	*ma*	*PN*	*IN*

This constitutes a perfectly regular 2 : 2 segregation of all genes. But nucleus 1 and 4 (and possibly 2 and 3 as well) each undergo an extra mitotic division, and in two of the subsequent spores there are incorporated two nuclei so that the final tetrad consists of the following spores (A, B, C, D) :

Spore A with nucleus 1 :	α	*G*	*ME*	*MG*	*MA*	*pn*	*in*
Spore B with nuclei 1+2 :	αα	*GG*	*MEme*	*MGmg*	*MAma*	*pnpn*	*inin*
Spore C with nuclei 3+4 :	*aa*	*gg*	*MEme*	*MGmg*	*MAma*	*PNPN*	*ININ*
Spore D with nucleus 4 :	*a*	*g*	*me*	*MG*	*ma*	*PN*	*IN*

Thus one will find by analysis the occurrence of 3 *ME* : 1 *me*, 4 *MG* : 0 *mg*, and 3 *MA* : 1 *ma*, while all the rest of the gene pairs segregate normally. This is exactly what Mundkur found, although he apparently did not determine the haploidy or diploidy of the 4 single-spore cultures arising from this irregular ascus. It is not known how 2 α-nuclei or 2 *a*-nuclei—*i.e.* 2 nuclei belonging to the same mating type—will behave at spore germination nor how the subsequent single-spore cultures will develop. If they fuse, it is possible that the culture, in spite of its diploid nature, will be able to invoke a mating response in a culture of the opposite mating type. One may even imagine a diploid αα-type producing a mating reaction both with a haploid *a*-type and with a diploid *aa*-type. If, on the other hand, the nuclei do not fuse, one can expect the development of a chimerical culture—*i.e.* one composed of two haploids of the same mating type. When such a chimera is analysed *in toto*, it is naturally the dominant genes that will express themselves, just as in a hybrid. Giant colonies should disclose whether a true chimera were involved, but it is not known whether Lindegren and Mundkur used giant colonies as controls.

The single tetraploid ascus of Roman, Hawthorne, and Douglas (1951) and the published results of Roman and Sands (1953) should be mentioned in this connection, since it is possible to explain these results on the assumption that binucleate spores occurred in their material, which was originally obtained from Lindegren. The authors are fully aware of this possibility, and offer this as an explanation of their results, especially with regard to diploid mating types of the formula αα and *aa*. However, neither analyses of 3 : 1 segregations nor cytological studies were attempted.

It would seem that the occurrence of binucleate spores would be a more probable explanation than the regular segregation of an

autotetraploid ascus nucleus which seems to be preferred by these authors.

4. CYTOLOGICAL VERIFICATION OF THE OCCURRENCE OF SUPERNUMERARY MITOSES AND BINUCLEATE SPORES

Even though much disagreement prevails among yeast cytologists, no difficulty is involved in obtaining stained preparations in which the number of nuclei can be observed in vegetative cells, asci during sporulation, and in the spores themselves. Lietz's cytological investigations (1951) seem to us to be the most satisfactory so far, and, in general, we can confirm his observations except for the fact that in our material we were unable to distinguish the chromosomes so clearly as Lietz's drawings indicate he was able to do.

We are compelled to mention the cytological studies of Subramaniam and his colleagues (Subramaniam, 1948, 1953; Rao and Subramaniam, 1953; and Thiagarajan and Subramaniam, 1954). As far as we can tell, their so-called "chromosomes" may at times be identical with nuclei, but apparently by employing an unsatisfactory technique (*e.g.* osmium vapour fixation), these investigators have often obtained other stainable bodies in their preparations—bodies which show a typical "Spiegelfärbung" or other evidences of poor technique. Subramaniam's contentions (1) that a fermenting cell is cytologically abnormal and incapable of survival, (2) that one should study the cytology of the yeast cell only from aerated cultures, and (3) that the transition from diplophase to haplophase has no connection with spore formation since the doubling and halving of the chromosome set is said to occur in the vegetative phase of the life cycle—these bear witness to a lack of understanding of the biology of the yeast cell. In addition, since Subramaniam and his colleagues have not undertaken a single genetic analysis of a yeast culture, nor have they undertaken any cytological investigations of the pre-sporulating phase of the life cycle, it is not surprising that work from their hands has become so misleading that they are not in agreement with any other investigators.

Excellent staining of chromatin material is obtained by fixation with Zenker's fluid followed by hydrolysis and staining with Feulgen or the still better Azure (DeLamater, 1951), but it is obvious that when the nucleus of such a stained yeast cell in the resting stage is *ca.* 0·5 μ in diameter there is not much hope of being able to observe the chromosomes in detail under the ordinary light microscope.

We found Zenker's fluid to be the best fixative, and we employed 1-day-old streak cultures as well as aerated cultures in a liquid medium.

Plate I, fig. 1 shows two nuclei each with an apparent doubleness (anaphase) in two haploid cells. Fig. 2 shows a haploid cell with its nucleus in the telophase; a yet anucleate bud has been formed. Fig. 3 shows a diploid cell with a bud, both of which are uninucleate. Fig. 4 shows a young binucleate ascus, and a young 4-nucleate ascus

is seen in fig. 5. In fig. 6 is shown an ascus with 4 uninucleate spores. Fig. 7 shows an ascus with 3 uninucleate spores ; the fourth nucleus is seen lying free in the ascus. All of these stages are normal and found in large numbers in our preparations.

Figs. 8 and 9 are photographs of the same ascus in different foci. Both figures show clearly a binucleate spore, the ascus itself containing, as the result of extra mitoses, two binucleate spores. Also in fig. 10 is seen a binucleate spore in an ascus. Figs. 11 and 12 show in different optical levels a non-synchronous nuclear division in a young ascus ; one nucleus is dividing and is in the telophase while the others are in the resting stage. Fig. 13 shows an 8-nucleate ascus, in which 5 nuclei are seen in focus. Finally, in figs. 14 and 15 may be seen in two optical levels 4 nuclei dividing synchronously ; the 8 daughter nuclei are arranged in 4 pairs, one of which is not clearly in focus.

It is thus evident that there is complete agreement between the cytological observations and the genetic demonstration of the occasional occurrence in the ascus of supernumerary mitoses and the subsequent formation of binucleate spores.

5. SUMMARY

In the tetrad analysis of yeast fungi (*Saccharomyces*) there are occasionally found deviations from the theoretically expected simple mendelian 2 : 2 segregation ratios in the asci of monohybrids.

It has been claimed (Lindegren, Mundkur) that these deviating tetrads constitute proof of the correctness of Winkler's conversion theory, but the deviating tetrads investigated here did not arise by gene conversion. Neither are they the result of polyploidy, cytoplasmic inheritance, nor mutation. They are formed as the result of the occurrence of supernumerary mitoses within the ascus, resulting in 5-8 nucleate asci which are capable of producing binucleate spores.

Binucleate spores usually yield diploid heterozygous single-spore cultures. When a 3 dominant : 1 recessive type is segregated out in an ascus instead of 2 *d* : 2 *r*, one of the 3 dominants is usually heterozygous.

It is shown also in Lindegren's yeast material, which normally gives rise to haploid single-spore cultures (our material diploidizes automatically owing to the presence of the *D* gene) that when a 3 *d* : 1 *r* segregation occurs, one of the three dominant types is always diploid and usually heterozygous ; this is due to the incorporation of 2 nuclei in the spore in question.

The deviating tetrads found in multiple heterozygous hybrids (Mundkur) may also be explained on the basis of the occurrence of binucleate spores.

Cytological investigations have confirmed the occurrence of supernumerary mitoses and binucleate spores within the ascus.

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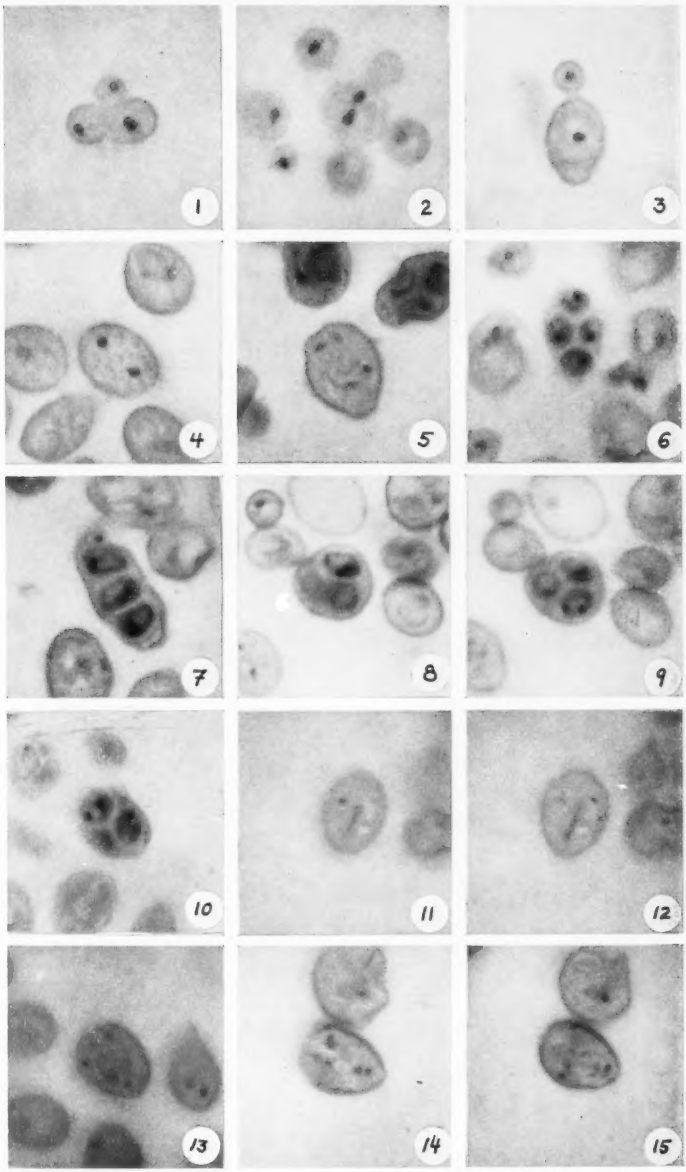
Plate I



Cytological evidence for the occurrence of supernumerary mitoses and binucleate spores in *Saccharomyces* (2700-2800 x). For explanation, see text, pages 302-303.

SELF-INCOMPATIBILITY SYSTEMS IN ANGIOSPERMS
II. *IBERIS AMARA*

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I. INTRODUCTION

SELF-INCOMPATIBILITY is known to be widespread in the Cruciferae and to include the economically important genus *Brassica*. No one has yet succeeded, however, in obtaining a coherent picture of the incompatibility system involved. In an earlier paper (1952) I have drawn attention to the need for a critical survey of angiosperm families to discover whether any hitherto undisclosed incompatibility systems were operating. It appeared therefore that the Cruciferae would repay closer study.

Three species have already been studied in some detail ; *Cardamine pratensis* (Correns, 1913), *Brassica oleracea* (Kakizaki, 1930) and *Capsella grandiflora* (Riley, 1936) and quite different mechanisms were invoked by the respective authors to explain the results in each species. Experience in the Solanaceae (*Nicotiana*, *Petunia*, *Solanum*), Scrophulariaceae (*Veronica*, *Antirrhinum*, *Nemesia*), Plumbaginaceae (*Limonium*, *Armeria*, *Acantholimon*, see Baker, 1948) and now Compositae (*Parthenium*, *Crepis*, *Cosmos*) indicates a uniformity in the self-incompatibility system operating in a given family. It appeared possible and desirable therefore to derive an incompatibility system which would fit all the data so far obtained in the Cruciferae.

In surveying the family for suitable experimental material I chose *Iberis amara*. Ironically, Darwin in " Cross and Self-fertilisation of Plants " describes it, together with *I. umbellata*, cabbage and radish, as self-fertile. *I. amara* is an annual which can be over-wintered if sown in autumn, but I have been unable to prevent it from dying after completion of flowering, so that it has not been possible to test the progenies against their parents.

(a) *Stigma flushing*.—This disadvantage is more than offset by the hundreds of flowers per plant and, especially, the flushing of stigma and style with purple anthocyanin following fertilisation, which seems to be a feature of the whole genus. This flushing is followed by the drying up of the stigma as it becomes un-receptive. An unpollinated or incompatibly pollinated stigma remains fresh, green and receptive indefinitely. Compatible pollen tubes grow down the style in about twelve hours. The flushing of the stigma has begun 24 hours after pollination and has developed the maximum intensity within 48 hours.

Incompatible pollen barely penetrates the stigmatic surface. This

early inhibition of the pollen has been observed in *Capsella* (Riley, 1932), Radish (Lewis, unpub.), *Brassica pekinensis* (Stout, 1931), and *B. oleracea* (Sears, 1937) and would therefore appear to be typical of Cruciferae. (A further indication that Crucifers have a common system.) The flush following compatible pollination enables one to carry out extensive series of pollinations duly determined by the reactions produced by pollinations of two days before. Also all unexpected or incomplete reactions can be repeated at once. Normally, five or six flowers are pollinated with any one cross, never less than three. It is necessary to carry out the pollination programme speedily because, as the plants become senescent, the flushing becomes tardier and weaker.

As might be expected, there is a strong correlation between the flushing of the stigma and seed setting. It is not complete however. Sometimes flushing is followed by failure to produce ripe seed. The first flowers produced on a plant are frequently incapable of forming ripe fruits. Sometimes a negative reaction is followed by partial setting, usually a half-fruit (each of the two carpels in *Iberis* contains one seed). This could be due to the slow growth of an only partially inhibited pollen tube. Since the stigma reaction is nearer to fertilisation than fruit development it is likely to be the more reliable of the two as an indication of compatibility.

It is worth noting that crosses between *Iberis amara* and other species such as *lagascana* and *sempervirens* produced the flushing just as readily as intraspecific pollinations (without producing any fruits), but no flushing followed crosses with more distant relatives such as species of *Lepidium*, *Sisymbrium*, *Erysimum* or *Arabis*. No observations were made on the distance travelled by the pollen tubes in these latter crosses. The sterility of all the species crosses confirms that the flushing of the stigma must be induced by a stage closely following fertilisation, if not by fertilisation itself.

Investigations into the mechanism were made in two series using material from different sources. The first series was exploratory. The second was more intensive, being based on the knowledge acquired from the first.

2. FIRST SERIES

The parent plants were grown in the spring of 1950 from seed supplied by the Helsinki Botanic Garden. Among the six plants (designated A-F) only the pollinations between A and the other five, in either direction, were compatible. Thus the six plants appeared to belong to two compatibility groups. Some seed was raised by selfing in the bud and selfing after cutting off the stigma, though neither produced an appreciable increase in seed setting.

Difficulty was encountered in that some families contained self-fertile plants, which were reciprocally cross-fertile with all other plants. The presence of self-fertile individuals did not reduce the

number of groups in a family so it was presumed that the self-fertility gene was not at the S-locus. There were also some male-sterile plants which could not be tested reciprocally. Sometimes, when self-fertility and male-sterility occurred in the same family, a male-sterile plant would be cross-fertile with all hermaphrodite sibs. In such cases it was presumed that these plants had the self-fertility genes masked by their male-sterility.

The original intention had been to make all possible pollinations within each family. But since, after making all pollinations, family 2 failed to disclose more than two groups, the procedure was modified as follows. One plant from each family was tested as female against all the rest. In general this revealed two groups, compatible and incompatible. A second plant from the same group as the first and

KEY TO FAMILIES

First sowing			Second sowing		
Family no.	Cross	No. of plants	Family no.	Cross	No. of plants
1	A×B	10	8	As (after decapitation of stigma)	1
2	recip.	17	9	B×D (?)	5
3	A×C	14	10	Bs (bud)	5
4	recip.	14	11	Bs (decap.)	0
5	A×D	6	12	Es (bud)	6
6	recip.	13	13	Es (decap.)	6
7	E×A	12	2	B×A (resowing)	11

two from the second group were tested again in the same way. In general this still only revealed two groups and it is doubtful in that case whether further *intra-family* pollinations would have yielded any more information. In the inter-family pollinations only one plant from each group was used and then not reciprocally (because there had previously been very few reciprocal differences : 2 out of 92 pairs of pollinations). Experience from the second series shows that it would have been better to have used three plants from each group in an attempt to include the two genotypes of each group in the pollinations, and to have tested all reciprocally.

(a) *General.*—After excluding the self-fertile plants the families generally contained only two mating groups. (The exceptions will be considered later.) With gametophytic determination of pollen (as in *Nicotiana*) this could only mean that the parents had one S-allele in common ; and in consequence reciprocal matings would yield unlike families :

$$S_1\ S_2 \times S_2\ S_3 \rightarrow S_1\ S_3 + S_2\ S_3$$
$$S_2\ S_3 \times S_1\ S_2 \rightarrow S_1\ S_3 + S_1\ S_2.$$

In *Iberis*, however, reciprocal matings yielded the *same* two groups.

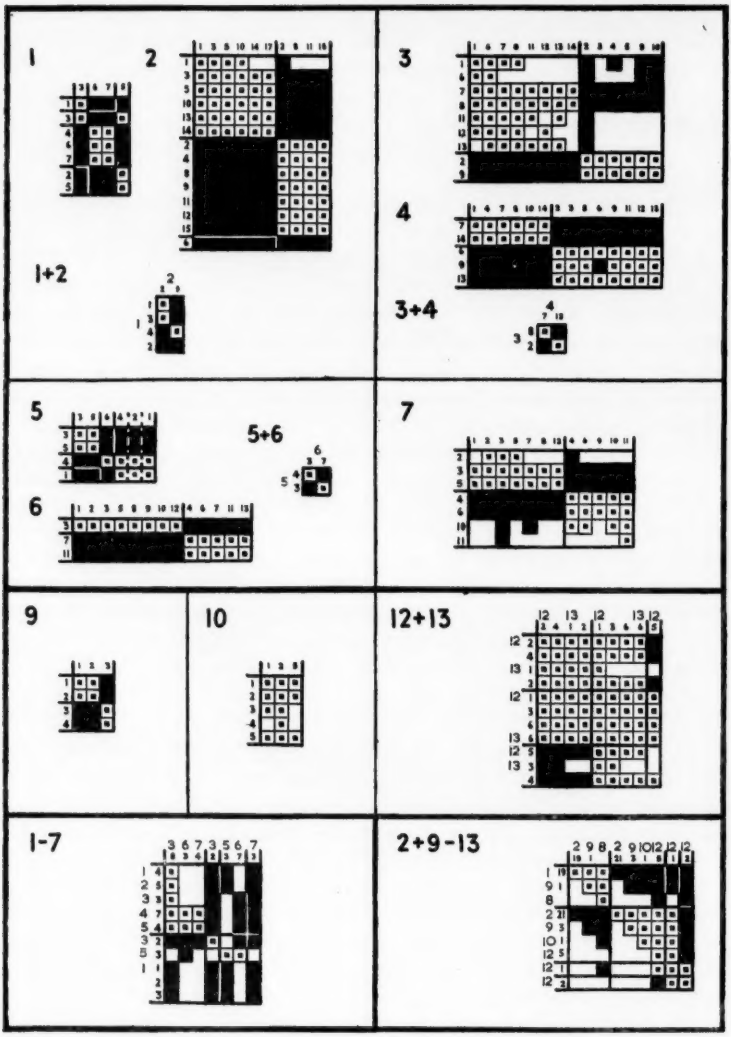


FIG. 1.—The results of all intra- and inter-family pollinations of the first series, excluding self-fertiles. Rows represent plants tested as females, columns plants tested as males. Plants have been grouped according to their behaviour. Symbols :

■ compatible pollination.
⊡ incompatible pollination.
Blank : pollination not made.

In family 5 it was not possible to classify plant 2, so it is separated from the two groups to which it might belong by broken lines.

The most critical evidence is from families 12 and 13 combined (E selfed). Of 12 plants 1 was self-fertile. The remaining 11 fell into three groups, so the plant E must have been heterozygous. One of the three groups was reciprocally incompatible with both the others. These were reciprocally cross-compatible (see fig. 1). The constitution of parent E is given as **S3 S7**. The three groups would then be

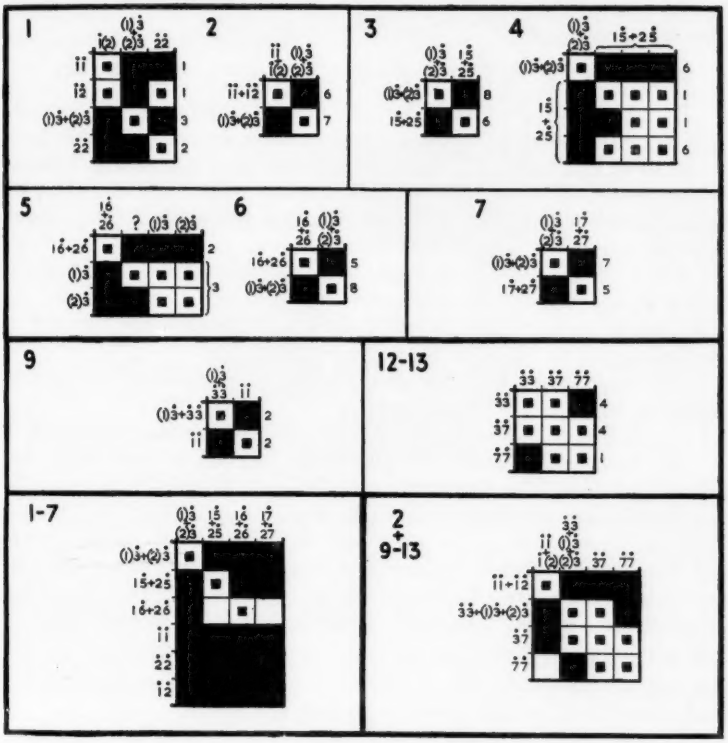


FIG. 2.—Interpretation of the results given in fig. 1. Each mating group is now represented by a single row or column with the genotype and phenotype (style and pollen) and frequency (to right of rows) indicated.

i indicates an active allele.
(i) an inactive (recessive) allele.
i no evidence.

S3 S3, **S7 S7** and **S3 S7** and *all* pollen of **S3 S7** was rejected by both **S3 S3** and **S7 S7**. The reaction of the pollen of **S3 S7** must be sporophytically determined with independent action of both alleles.

The two groups in each pair of reciprocal families could be due to the homozygosity of common parent A. The evidence of family 1, apparently contaminated by seed of A selfed, indicates however, that A was heterozygous and that the two mating groups of the other

families hide four genotypes whose identity is hidden by dominance. Inter-family pollinations revealed that there was one group common to all the families, except 8, which (being A selfed) differed from all the rest in not being descended from one of the inter-sterile parent plants B-E. Evidently this group had in common the S-allele which produced the cross-incompatibility between the parents B-E.

In general the results are consistent with the following parental genotypes :—

A, S1 S2 ; B, S1 S3 ; C, S3 S5 ; D, S3 S6 ; and E, S3 S7.

In all further discussion, for the sake of clarity, the S symbols will be omitted and the genotypes will be represented by the numbers alone in heavy type. The activity of an allele is denoted as follows. Where proven to be active the number of the allele has a dot over, $\dot{3}$: when proven to be inactive (recessive) it is in parenthesis, (3) ; otherwise the number is unqualified, 3.

The evidence both of genotype and of activity of individual alleles is not as unambiguous in this series as in the second series and I do not propose to discuss it further. A full treatment of the method of deduction will be given for the second series.

The interpretation of the results of fig. 1 in terms of S-alleles is shown in fig. 2.

(b) *Activity of* S-*alleles*.—The data can be summarised as follows :

We have encountered 16 genotypes and have discovered the following information about their constituent alleles. One genotype has been shown to behave differently in pollen and style : the phenotype of parent A is $\dot{1}.\dot{2}$ in style and $\dot{1}.(2)$ in pollen. Seven other genotypes (four of them homozygous) are known to behave alike in pollen and style : $(1).\dot{3}$, $(2).\dot{3}$, $\dot{3}.\dot{7}$, $\dot{1}.\dot{1}$, $\dot{2}.\dot{2}$, $\dot{3}.\dot{3}$, and $\dot{7}.\dot{7}$. The remaining 8 genotypes have not been fully tested and it is not known whether the style and pollen reactions are the same : $\dot{3}.5$, $\dot{3}.6$, $1.\dot{5}$, $2.\dot{5}$, $1.\dot{6}$, $2.\dot{6}$, $1.\dot{7}$ and $2.\dot{7}$. We know that at least one of the alleles 1 and 2 is recessive in the pollen to each of 5, 6 and 7.

If we assume that alleles which are independent of each other will bear similar dominance relations to further alleles (which by analogy with step-allelomorphs is to be expected and might be used as a working hypothesis) we can place the alleles in a descending order of dominance.

Then in the style $3 = 7 \doteq 5$ and $6 > 1 = 2$.
and in the pollen $3 = 7 \doteq 5$ and $6 > 1 > 2$.

(c) *Conclusions*.—The experiment has established the following facts. Incompatibility in *Iberis amara* involves sporophytic control of the pollen as well as the style. There are several alleles, 5 plants from a botanic garden population (probably very small) sharing 6 alleles between them (one of the original 6 plants was not represented in the progenies). Pairs of alleles can show independence or dominance,

in either pollen or style. The dominance relations of a series of alleles show a strong but not complete correlation between pollen and style. This lack of correlation is reflected in 2 out of 92 pairs of reciprocal pollinations being discordant.

Self-fertility seems to be fairly frequent, the breakdown of incompatibility appearing to occur equally on the male and female sides for pollinations between self-fertile and self-sterile plants are reciprocally compatible. In this material the self-fertility is not due to a change at the S-locus.

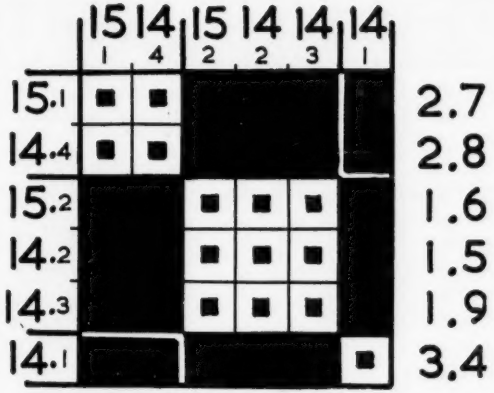


FIG. 3.—Compatibility relationships of the parent plants of the second series. There are three cross-compatible classes and no reciprocal differences. Proposed genotypes entered at right of rows.

The reliability of the technique can be judged by the fact that out of 552 different cross-pollinations only one ($4^9 \times 4^6$) was inexplicable.

3. SECOND SERIES

A second series of crosses was made in 1951, to test whether the conclusions from the first series applied in unrelated material, and to obtain more extensive information on the dominance and other inter-relations of a sample of S-alleles.

The parental material was obtained from Poznan Botanic Garden in two seed packets : one labelled *I. amara*, sown as family 14, the other *I. amara alba odorata*, sown as family 15. The two packets produced very similar plants which showed as much cross-incompatibility between packets as within packets, so they probably came from the same population. Their compatibility relationships are shown in fig. 3.

Assuming sporophytic control, plants 15.2, 14.2, and 14.3 must have a common allele, say S1 * and plants 15.1 and 14.4 must also

* The numbering of the alleles in the second series is quite independent of that of the first series. There was no cross testing.

have had a common allele, say **S2**. The maximum number of alleles shared among the six plants is therefore nine. The other seven alleles have been numbered **S3** to **S9**, the final numbers being allocated in

TABLE 1

Origin and constitution of families 16-35

Family number	Number of plants	Parent numbers	Parent genotypes	Genotypes expected in progeny	Proportion of self-fertiles
16 17	10 10	14.1 × 14.2 recip.	3.4 and 1.5	1.3 : 1.4 : 3.5 : 4.5	0/20
18	8	14.1 × 14.3	3.4 and 1.9	1.3 : 1.4 : 3.9 : 4.9	4/8
19 20	7 8	14.1 × 14.4 recip.	3.4 and 2.8	2.3 : 2.4 : 3.8 : 4.8	4/15
21 22	10 10	14.1 × 15.1 recip.	3.4 and 2.7	2.3 : 2.4 : 3.7 : 4.7	0/20
23 24	8 10	14.1 × 15.2 recip.	3.4 and 1.6	1.3 : 1.4 : 3.6 : 4.6	0/18
25	12	14.2 × 14.4	1.5 and 2.8	1.2 : 1.8 : 2.5 : 5.8	5/12
26 27	10 10	14.2 × 15.1 recip.	1.5 and 2.7	1.2 : 1.7 : 2.5 : 5.7	0/20
28 29	9 7	14.3 × 14.4 recip.	1.9 and 2.8	1.2 : 1.8 : 2.9 : 8.9	2/16
30 31	5 2	14.3 × 15.1 recip.	1.9 and 2.7	1.2 : 1.7 : 2.9 : 7.9	1/7
32 33	10 10	14.4 × 15.2 recip.	2.8 and 1.6	1.2 : 1.8 : 2.6 : 6.8	6/19 *
34 35	10 6	15.1 × 15.2 recip.	2.7 and 1.6	1.2 : 1.7 : 2.6 : 6.7	0/15 †

* The 20th plant was feeble, male-sterile, and was not tested.

† One plant appeared to be male-sterile and "self-fertile" (see text).

accordance with their position in the dominance series as indicated by the experiment.

We can predict the genotypes to be found in all the families produced by intercrossing the parent plants. These are shown in table 1 together with the family numbers and the number of plants per family. The proportions of self-fertile plants are also shown.

Evidence presented later will indicate that these self-fertile plants (in contrast with those of the first series) were so by virtue of their S-constitution. We can in any case be sure that none of the genotypes present in entirely self-sterile families are self-fertile and thus by a process of elimination can arrive at those few genotypes which might be self-fertile.

We can thus classify all genotypes according to their self-sterility and according to the families in which they will be expected.

TABLE 2

Classification of genotypes according to the families in which they are expected

Genotype	Family	Genotype	Family
Found in wholly self-sterile families : *i.e.* self-sterile			
1.2	25, 26-7, 28-9, 30-1, 32-3, 34-5	2.7	(Parent 15.1)
1.3	19-20, 21-2	2.8	(Parent 14.4)
1.4	19-20, 21-2	3.4	(Parent 14.1)
1.5	(Parent 14.2)	3.5	16-7
1.6	(Parent 15.2)	3.6	23-4
1.7	26-7, 30-1, 34-5	3.7	21-2
1.9	(Parent 14.3)	4.5	16-7
2.3	16-7, 18, 23-4	4.6	23-4
2.4	16-7, 18, 23-4	4.7	21-2
2.5	25, 26-7	5.7	26-7
2.6	32-3, 34-5	6.7	34-5
Found only in partially self-fertile families : *i.e.* possibly self-fertile			
1.8	25, 28-9, 32-3	4.9	18
2.9	28-9, 30-1	5.8	25
3.8	19-20	6.8	32-3
3.9	18	7.9	30-1
4.8	19-20	8.9	28-9

It must be remembered that nine is only the maximum possible number of alleles. It is quite likely that the actual number was less, and that different numbers cited above and in table 1 refer to identical alleles.

(a) *Intra-family tests.*—Each family consisted of ten plants when possible. The method of making test pollinations was slightly modified from the first series since if four genotypes are expected in a family one must try as far as possible to recognise all four of them. The more rough and ready methods used in the first series had not fully tested or recognised all genotypes. After putting self-fertile plants to one side one plant of the remainder from one of each pair of reciprocal families was tested as ♀ against its sibs. As a rule some were compatible, making a second class. When no compatibility was disclosed (*e.g.* family 25) two further plants were tested. One plant in any new class was at once tested as seed parent against the remainder. This usually revealed at least one and usually two further classes which were again tested as ♀♀.

The next step was to check that the reciprocal had the same four classes by testing against the four test plants of the first family. Agreement was the rule. In family 24 only two classes had been found and

a third turned up in the reciprocal 23. This in its turn when used as a tester on 24 revealed the fourth class. The four final classes behaved as expected. Details of these intra-family pollinations are shown in fig. 4.*

It will be noticed that crosses between identical S-genotypes may be compatible and half-compatible (21-2, 26-7), or incompatible and half-compatible (26-7, 28-9). This is not surprising in view of the delicate balance in strength of reaction needed to produce half-compatibility. Slight variations in modifiers could easily swing it over from full setting to no setting.

In only two pairs of reciprocal families was there any discordance. Family 16 and the reciprocal 17 each had four classes but two of those in 17 were represented by single plants neither of which corresponded to one of the four in 16. The other two classes were represented in both. One plant from each group in 16 had already been entered for the inter-family crosses and the aberrant plants in 17 were not tested further. However, the four test plants from 16 all behaved appropriately so we can assume that the anomalous plants in 17 were contamination of some kind.

The only other family for which it was necessary to invoke contamination was 34-5. On first examination there appeared to be four mating groups between them, two comprising single plants. One of these, 34.3, was more or less sterile with all other groups when tested as ♂, but compatible with all as ♀. Closer examination showed that though the anthers were of normal size they were empty of pollen. Its behaviour could be explained as that of a self-fertile but male sterile plant (a constitution frequently met with in the first series). Later tests showed 34.1 to be **1.2** with both alleles active. Then 34.7 must be **6.7**, with **1.7** and **2.6** as the missing genotypes, both of which are already known to be incompatible with **1.2** (34.1). Therefore 34.2 does not belong to any one of the four expected groups and is probably a stray seedling. On the other hand **1.7** and **2.6** might well be compatible with **6.7**, in which case from the pollinations shown in fig. 4 they would be classified with 34.1 giving an expected ratio of 3 : 1 ; the actual ratio is 10 : 4.

The following families had four self-sterile classes (exclusive of presumed contaminants) : 16-7, 21-2, 23-4 and 26-7. These contained no self-fertile plants.

Of the remainder, the following had three self-sterile and one self-fertile class : 19-20, 28-9, 30-1 and 32-3. This in itself is a strong indication that the self-fertility (except in 34.3 ?) was due to a particular S-allele constitution.

* In addition to the two types of pollination represented in figure 1, there is a third in which the square is half blacked out. This has no connexion with the intensity of the stigma coloration which is quite variable but characteristic of the seed parent. It means that only a proportion of the pollinated stigmata flushed. These results were repeatable. They must be due to weak compatibility. In the wild, with open pollination, such weak compatibility would be effective incompatibility.

Confirmation of this is given by the fact that in a family produced by crossing unrelated plants (though not in one produced by a compatible cross between plants with a common allele) each incompatibility class out of the four will be compatible with at least one

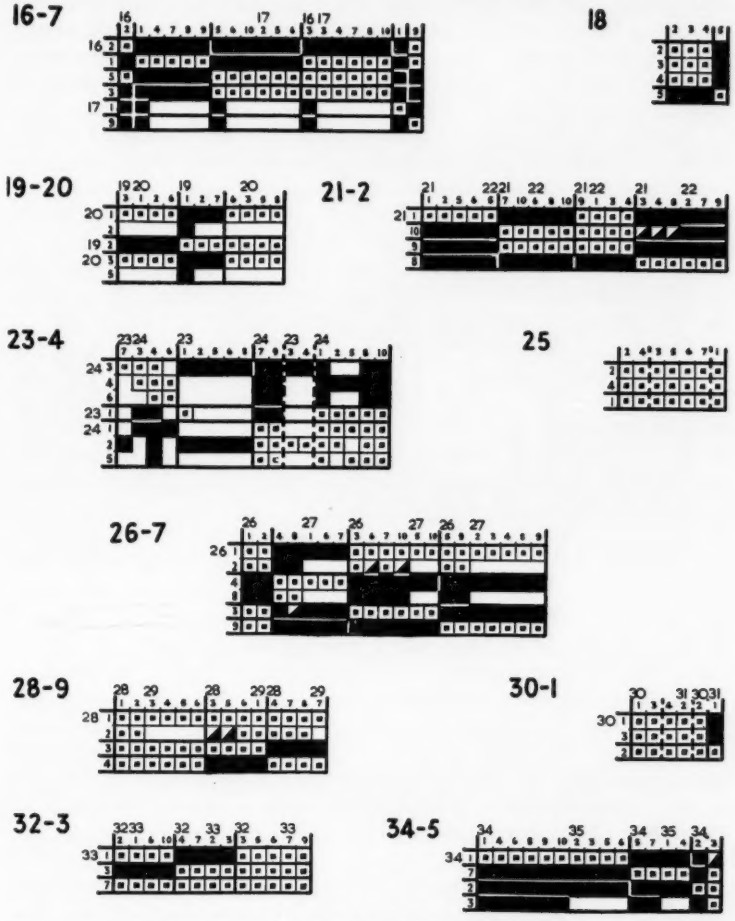


FIG. 4.—Second series. The results of all intra-family pollinations, excluding self-fertiles. Plants have been grouped according to their behaviour. In addition to the symbols of fig. 1 we have :

◪ weakly incompatible.

other, the complementary one with no common allele. Reference to fig. 4 shows that in families 19-20, 28-9 and 32-3, the pollen of one of the self-sterile classes was incompatible with all the self-sterile plants. Similarly in families 28-9, 30-1 and 32-3 the style of one of

the self-sterile classes was incompatible with all the other self-sterile plants. *The complementary genotype must have been the self-fertile class.*

The following families had less than four classes revealed.

Family 18 had only eight plants and was without a reciprocal. Four plants were self-fertile. The remaining four fell into two classes but one of the expected classes, 1.3, which was known to be self-sterile, was missing from this family. Thus instead of an expected four equal classes we had 3 : 1 : 0 (self-sterile) : 4 (self-fertile), not an unlikely ratio in a small family.

Family 25 was again without a reciprocal but contained twelve plants of which five were self-fertile. The remaining seven appeared to comprise one class since they were all incompatible with each other. However, just in case two genotypes were present three plants were entered for the inter-family tests. These showed them to comprise two classes, the third self-sterile class being missing. Instead of an expected ratio of 2 : 1 : 1 (two of the classes not being separable) the actual frequencies were 7 : 0 : 5 (self-fertile).

Family 34-5 has already been considered.

The cross-relationships of the classes within a family can give only limited information about the gene actions and give no clue to the identity of the different alleles. Their main value is in the detection of the classes. For further understanding we need inter-family pollinations. (Backcrossing to the parents would be equally informative but is impossible in this annual species.)

(b) *Inter-family tests.*—One plant was selected from each self-sterile class in a reciprocal pair of families (more than one when a class was suspected of containing more than one genotype) and each was tested against all the other families—thirty-eight plants in all were used. Of those families containing four classes, in 23-4 and 30-1 one of the self-sterile classes was disclosed too late to be included in the tests.

The first nine test plants were crossed as female to every other one. As will be expected from table 2, many genotypes were represented several times. In order to save superfluous work test plants behaving alike and coming from families with a common genotype were reduced to one representative. Unfortunately this sometimes resulted in discarding plants with similar phenotypes but different genotypes and consequently other genotypes remained over represented. Altogether sixteen original test plants were eliminated half-way through, leaving twenty-two. The number of pollinations required was thus reduced from 38^2 (1444) to 22^2 (484). The behaviour of the thirty-eight test plants when pollinated on to the first nine was found to fall into a limited number of patterns. One of these can be excluded : the partially male-sterile 34.3. The remaining thirty-seven plants show only 12 behaviour patterns, some very common, some unique. They are summarised in fig. 5. The patterns are numbered (i) to (xii) and below each are listed the test plants showing that pattern.

It will be seen that the incompatibilities shown by some plants

are the sum of those shown by others. Thus (iv) has the incompatibilities of (i) and (ii), (v) those of (i) and (iii) and (vi) those of (ii) and (iii). This can only mean that groups (i), (ii) and (iii) each have an active incompatibility allele and that groups (iv), (v) and (vi) have the same alleles active in pairs. If we examine the families represented in group (v) and compare them with the data of table 2 we will see that all these families have one common genotype, **1.2**, in which both alleles must be active in the pollen. For the same reasons group (iv) must be of constitution **2.3** or **2.4** and group (vi) **1.3** or **1.4**. It will be noted that, formally, one cannot distinguish between

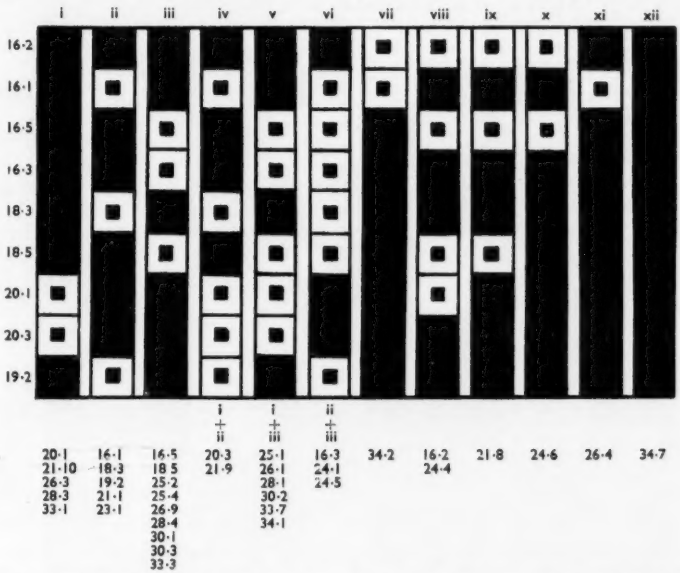


FIG. 5.—Thirty-eight inter-family test plants used as ♂♂ are grouped according to their twelve different reactions with 9 test plants used as ♀♀. *N.B.* The incompatibilities of groups (iv), (v) and (vi) are the sums of the compatibilities of groups (i), (ii) and (iii) in pairs.

3 and **4** because as they are the two alleles in plant 14.1 they will always occur as alternate alleles in any family of which it is a parent. We can therefore arbitrarily attribute the genotype **2.3** to (iv) and **1.3** to (vi). By an extension of this argument (i) has **2** active in the pollen ; (ii) has **3** active and (iii) has **1** active. (It will be noted that we have obtained this information *without reference* to the genotypes of the nine tester plants used as females.)

From this starting point we are able to make an almost complete analysis of the whole of the data in the inter-family crosses. Thus, in the family 19-20 we expect the genotypes **2.3**, **2.4**, **3.8** and **4.8**. We know that 20.1 has **2** active in the pollen, 19.2 has **3** active and 20.3 is **2.3**. Therefore 20.1 must be **2.4**, 19.2 must be **3.8** and the

self-fertile plants will be the fourth genotype 4.8. Having determined the genotype of all our test plants in this way we can establish which alleles are active in which combinations. The data from the twenty-two tester plants intercrossed in all ways is shown in fig. 6. The plants have been arranged according to their behaviour, and the conclusions as to the behaviour of the S-alleles in pollen and style are shown at the bottom of the columns and the left of the rows. Sometimes an allele is in an intermediate condition, not acting at

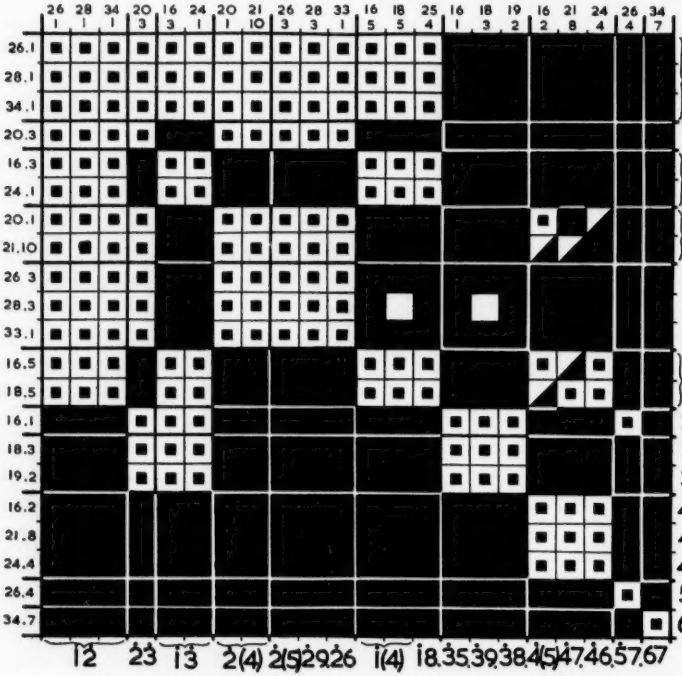


FIG. 6.—The 22 inter-family test plants intercrossed in all possible ways (except 25.4 which was not used as a ♀) and grouped according to their behaviour. At the end of the row or column is given the genotype and gene activities inferred from the results. In addition to those symbols used in fig. 2, (4̇) indicates weak activity.

sufficient strength to cause full incompatibility. It is then shown thus : (4̇). Where the information is insufficient the symbol is unqualified.

(c) *Method of inference.*—Extreme caution must be used in interpreting the data since the activity of the alleles in style and pollen *may* be quite independent. Having found an adequate explanation of the data one is tempted to cease searching for alternatives. But we are here trying to obtain definite information about the interaction of alleles and therefore we must allow for all types of gene action not excluded by the data.

If we confine our attention to the intra-family pollinations which have disclosed four classes, it is usually possible to arrange the 4×4 pattern of compatibilities so that whereas the top left to bottom right diagonal (representing the intra-class pollinations) is fully incompatible, the other diagonal is fully compatible. This is because in families containing four alleles it is always possible to group the four classes into two pairs which have no common allele and are thus inevitably cross compatible. The fully compatible diagonal is obtained when the two innermost and two outermost pairs of classes are without a common allele. Thus :

Conversely, if a 4-group, 4-allele family is arranged so as to give a compatible diagonal, it is probable that the inner and outer pairs

TABLE 3

	ab	bc	ad	cd
ab	–	?	?	
bc	?	–		?
ad	?		–	?
cd		?	?	–

always gives a compatible diagonal

but

	ab	cd	bc	ad
ab	–		?	?
cd		–	?	?
bc	?	?	–	
ad	?	?		–

will only give a compatible diagonal if there is a high degree of cross-compatibility in the family.

of groups are complementary, unless the degree of cross-compatibility between groups is high. An incorrect diagnosis will probably be recognised during later cross-testing.

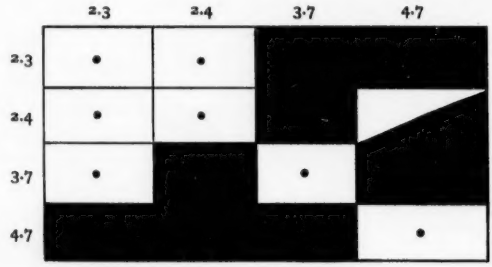
An important exception to the rule of the compatible diagonal arises when the parents had one allele in common so that the four genotypes in the family are produced by only three alleles. One of the classes may then be incompatible with the other three classes. On the other hand, if it is compatible with one of the other classes, it will be possible to produce a compatible diagonal, but further analysis on the assumption that one is dealing with a four-allele family instead of in fact a three-allele one will be misleading. That is obviously one trap to beware of in such analyses. Further data in the form of inter-family tests or backcrosses should reveal the true situation.

Having determined which pairs of genotypes have no common allele one can arbitrarily designate the alleles **a b c** and **d** and examination of the eight interclass pollinations not on the diagonals will give information of their activities. All eight pollinations are between classes with one common allele. If any are incompatible then the common allele must be active in both the style of one and the pollen of the other. If any such crosses are compatible one can reach a

definite conclusion only if the common allele has already been shown to be active in one of the classes. It must then be inactive in the other. If one allele has shown itself inactive in either style or pollen, the other allele must be active in both style and pollen of every self-incompatible genotype. But if two classes with a common allele of hitherto unknown activity are compatible, the allele may be inactive in style of one, pollen of the other, or both.

Let us now make such an analysis on family 21-2 whose interclass compatibilities are shown in fig. 7, arranged so as to give a compatible diagonal. For the argument to be followed more easily it is reproduced here.

TABLE 4



Excluding the diagonals, examination of the incompatible pollinations enables us to conclude that 2 is active in both style and pollen of 2.3 and 2.4 and that 3 is active in style of 3.7 and pollen of 2.3.

The partial incompatibility of pollination $\dot{2}.4 \times 4.7$ needs special consideration. In detail, a $\dot{2}.4$ plant, pollinated by six 4.7 plants gave full compatibility with three but only partial compatibility with the other three. Thus 4 either in style of $\dot{2}.4$ or pollen of 4.7 or in both is not acting at full strength but we know nothing more definite.

Our conclusions so far are summarised as follows :

Pollen . .	$\dot{2}.3$	$\dot{2}.4$	3.7	$\overset{?}{4.7}$
Style . .	$\dot{2}.3$	$\overset{?}{\dot{2}.4}$	$\dot{3}.7$	4.7

Let us now turn to the compatible pollinations.

All that we are able to conclude from the compatible pollinations $\dot{2}.3 \times 3.7$, $4.7 \times \dot{2}.4$, 4.7×3.7 and $\dot{3}.7 \times \overset{?}{4.7}$ is that 3 is inactive in style of $\dot{2}.3$, pollen of 3.7, or both ; 4 is inactive in the style of 4.7, pollen of $\dot{2}.4$, or both ; 7 is inactive in style of 4.7, pollen of 3.7, or both, and also inactive in style of $\dot{3}.7$, pollen of $\overset{?}{4.7}$, or both.

We can however go a stage further because, since 4.7 is self-sterile, 4 and 7 cannot *both* be inactive in the style.

So we have made a little progress. But we can still draw on information from the inter-family pollinations of fig. 6, which show

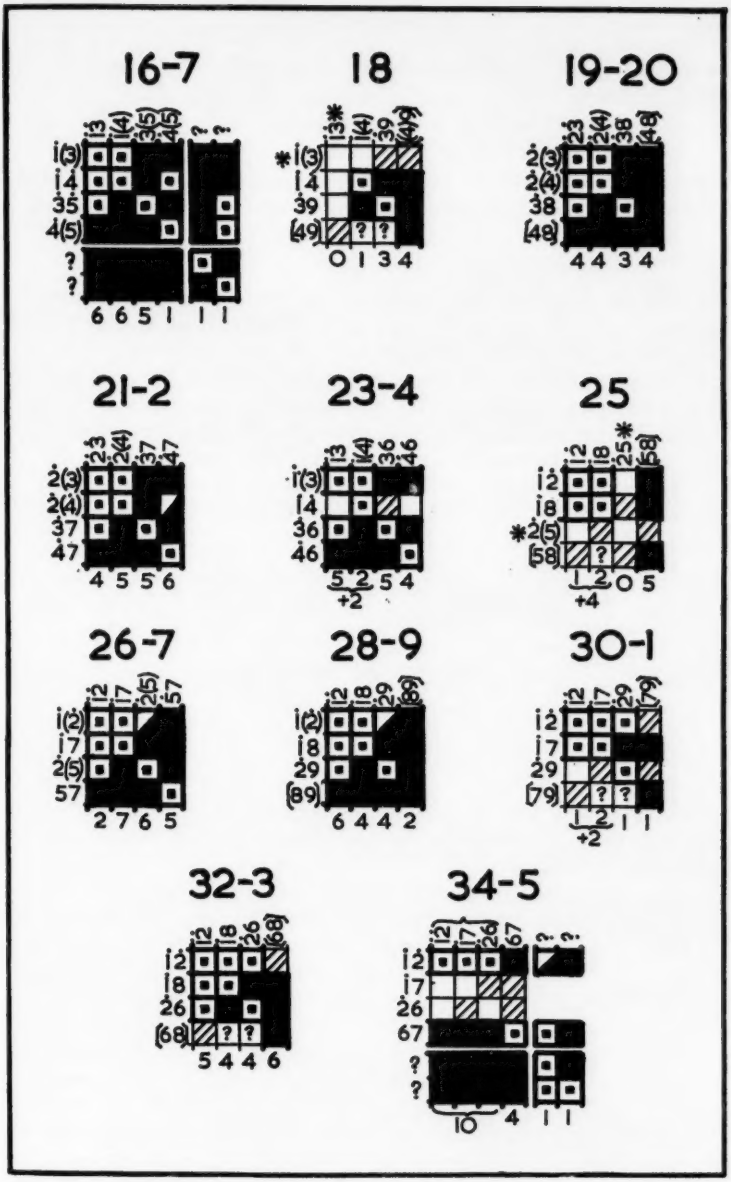


FIG. 7.—A summary of all class compatibilities within families, giving their genotypes, activities and frequencies (at foot of columns). In addition to the symbols of figs. 4 and 6 are the following :

[58] self-fertile ;

□ pollination not made but presumed incompatible ;

■ pollinati n not made, but presumed compatible ;

|?| pollination not made, compatibility not predicted ;

* genotype expected but absent.

us that 4 is active in pollen and style of $\dot{4}.7$. We are at once able to conclude that

(*a*) 4 in style of $\dot{2}.(\dot{4})$ is almost inactive
(*b*) 4 in pollen of $\dot{2}.(4)$ is inactive.

From the same source we find that 3 is inactive in the style of $\dot{2}.(3)$. Our whole knowledge of this family can now be summarised thus :

Pollen . . .	$\dot{2}.\dot{3}$	$\dot{2}.(4)$	3.7	$\dot{4}.7$
Style . . .	$\dot{2}.(3)$	$\dot{2}.(\dot{4})$	$\dot{3}.7$	$\dot{4}.7$

To this we may add that some of the remaining alleles must be inactive. Not all can be, for since 37 is self-sterile, either 3 or 7 must be active in both pollen and style.

The interpretations of all intra-family results of fig. 4, based on similar analyses, are given in fig. 7. Self-fertile genotypes are placed entirely in parenthesis, but this must not be taken to imply that both alleles are necessarily inactive in the pollen *and* style.

(d) *Second generation.*—Further evidence was obtained from a second generation raised from all compatible crosses within family 19-20. These were :

TABLE 5

Cross	Family number	Parental genotypes	Expected progeny genotypes							
$19^1 \times 19^4$	36	3.8×4.8	3.4	3.8	4.8	8.8				
19^4 s,	37	4.8			4.8	8.8	4.4			
$20^2 \times 19^1$	38	2.4×3.8	3.4		4.8			2.3	2.8	
$20^2 \times 19^4$	39	2.4×4.8			4.8		4.4		2.8	2.4
$20^5 \times 19^4$	40	2.3×4.8	3.4	3.8					2.8	2.4
$20^5 \times 19^1$	41	2.3×3.8		3.8				2.3	2.8	3.3

In addition, as a control, a re-sowing was made of family 21, which also served as a check on the peculiar reciprocal differences in behaviour of 3 and 4.

Fig. 8 shows the results obtained in the intra-family pollinations, together with expectations based on the previous generation. In general, agreement is very good. Of special importance is family 37 produced by selfing the self-fertile genotype 4.8 which segregates a single self-sterile group as a recessive. This is 4.4, showing 8 to be a self-fertility allele at the S-locus.

New evidence is obtained on the genotype 3.4. The inability to distinguish 3.4 from 3.8 in families 36 and 40 shows that 3 must be active in pollen and style though the occasional half-compatibility of some pollinations indicates a possible weakening of 3, probably in the style. The activity of 4 in 3.4 was tested by special crosses using 4.4 plants from family 37 and 3.4 plants from family 38 the only one in which this genotype could not be confused with 3.8. The crosses were compatible on to 4.4 styles but only partially compatible on to 3.4 styles. Here 4 is recessive to 3 in pollen but only partially so in style.

The activity of 4 was also tested in self-fertile 4.8 plants from family

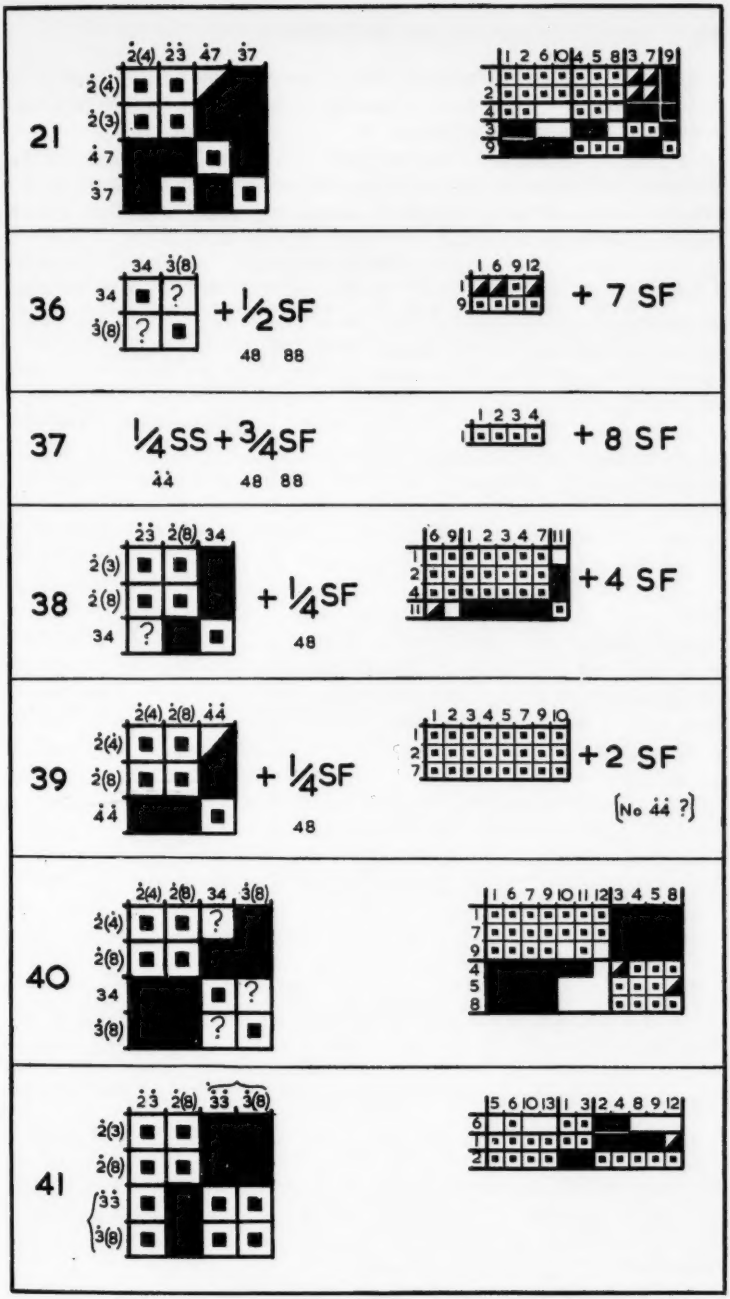


FIG. 8.—The expected genotypes and their reactions in the second generation (left) compared with the actual results (right).

39 using 4.4 plants as testers. This showed 4 to be fully active in style but inactive in pollen. Thus self-fertility does not exclude the unilateral activity of S-alleles.

(e) *Gene interactions.*—The evidence for gene interactions in the various heterozygotes varies greatly in extent. The weight of the evidence for all those genotypes in which something is known of both alleles is shown in table 6.

TABLE 6

The number of pollinations showing full activity, weakness or recessivity of the two alleles in those heterozygotes in which the behaviour of both alleles is known

Genotype	Allele	Style			Pollen		
		Fully active	Weak	Recessive	Fully active	Weak	Recessive
1.2	1	39	...	...	38	...	...
	2	37	4	...	31	...	...
1.3	1	35	...	...	18	...	...
	3	...	...	31	20	...	...
2.3	2	24	...	...	27	...	...
	3	...	...	20	21	...	...
1.4	1	38	...	...	22	...	...
	4	6	2	1	...	...	18
2.4	2	32	...	...	25	...	...
	4	1	10	6	...	...	18
3.4	3	*	1	...	*	...	...
	4	1	3	...	...	...	3
2.5	2	10	...	...	8	...	...
	5	...	...	4†	...	...	1†
3.5	3	15	...	...	2	...	...
	5	1†	...	...	?	?	?
4.5	4	4	...	...	6	...	...
	5	...	...	1†	...	...	1†
4.f	4	3	...	...	...	...	4

1.f, 2.f, 3.f. Recessivity of f on both sides is proved by the self-sterility of these genotypes.

* Failure to distinguish 3.4 from 3.8 in families containing both, indicates activity of 3 with possibly some weakening.

† All evidence with this sign traces back to a single pollination 5.6 × 3.5 which was incompatible. Therefore little weight can be attached to it.

Out of a total of 45 possible combinations only 6 homozygotes and 4 heterozygotes are entirely unknown. Of a large number all we know is that one of the alleles is active. Only 19 phenotypes are thoroughly analysed.* Of these, 13 are alike in pollen and style (four being homozygotes without the possibility of interaction) and the remainder are more or less dissimilar.

* Note the paradox that since the phenotype is so complex it is easier to determine a plant's genotype than its phenotype.

The most striking features are the contradictory dominance relations of 3 and 4. Allele 3 is active in the pollen of genotypes 1.3 and 2.3 but recessive to 1 and 2 in the style. On the other hand 4 is active in the style of genotypes 1.4 and 2.4, though not always strong enough to give complete incompatibility, and recessive to 1 and 2 in the pollen. These genotypes have been studied from a large number of pollinations so that there can be no doubt of the validity of these assertions. Particular interest therefore attaches to the activity of the alleles in 3.4 plants. The evidence of the second generation shows that 3 is active in pollen and style, whereas 4 is active only in the style and then imperfectly. Since 4 is active in styles of 1.4 and 2.4 while 3 is recessive to 1 and 2 one might have expected 4 to be dominant over 3 in style of 3.4 making that genotype self-fertile. This is not so.

(f) *Self-fertility.*—It will be noticed that evidence is most easily obtained about those alleles which are frequently active. Where alleles are usually recessive there are no pollinations available to prove their activity in any combination where they might be active except by producing them in the homozygous condition. This applies in particular to the self-fertile genotypes. In two of the families, 19-20 and 28-29, the self-fertiles were tested in all combinations against their sibs but they gave no information about the activity of the alleles in pollen or style because the alleles concerned were recessive or partially recessive in the self-sterile sibs. This was rectified in family 19-20 by studying the second generation. Over all the self-fertile genotypes the only definite conclusions are about 4.8, described above, and 4.9 in family 18 where, since $\dot{1},\dot{4} \times 4.9$ is compatible, 4 must be inactive in the pollen. It may be pointed out that inactivity of both alleles in style *or* pollen or reversed dominance (*e.g.* (4).$\dot{9}$ in pollen and $\dot{4}$.(9) in style) would be sufficient to produce full self-fertility. There is no evidence so far that would rule out reversed dominance since there is a certain independence of gene action in pollen and style. Genotype 3.4 though self-sterile comes very close to being self-fertile through reversed dominance. Self-fertility due to homozygosity for a recessive self-fertility allele is ruled out in the case of 4.8. But 5 is known to be active in some genotypes and 6.7 is self-sterile, proving that either 6 or 7 or both are not self-fertility alleles. Thus, of the five self-fertile genotypes, 4.8, 4.9, 5.8, 6.8, 7.9 and 8.9, only two, 8.9 and 6.8 *or* 7.9, could be homozygous for a self-fertility allele.

This has a bearing on the number of S-alleles in this material, of which the maximum number was 9. We know that all the alleles shown to be active (1, 2, 3, 4 and 5) are different. The above evidence of the self-fertiles shows that 8, 9 and 6 or 7 could be the same self-fertile S-allele. But whilst 4.8, 4.9, 5.8, 6.8 and 7.9 are self-fertile 4.6, 4.7, 5.7 and 6.7 are self-sterile. Thus neither 6 nor 7 can be identical with 8 or 9, but 8 and 9 are probably identical self-fertility alleles.

We may rename them **f**. The simplest explanation therefore is that **8** and **9** are the same self-fertility allele (**f**) which is dominant to **4**, **5** and **6** (= **7**?) and recessive to **1**, **2** and **3** in the pollen, but recessive to at least **1**, **2**, **3** and **4** in the style and possibly to **5**, **6** (and **7**) as well. Thus the number of alleles in this material, originating from six plants, is 7 or 8. Their dominance relations are summarised in fig. 9. The connecting lines between alleles denote what is actually known about their relationships. Though **6** and **7** are unconnected with the other alleles we know they must be low in the dominance series because they are recessive in either style or pollen, or both, to all the others with the exception of **5** whose relation with it is not known. They are therefore placed at the bottom.

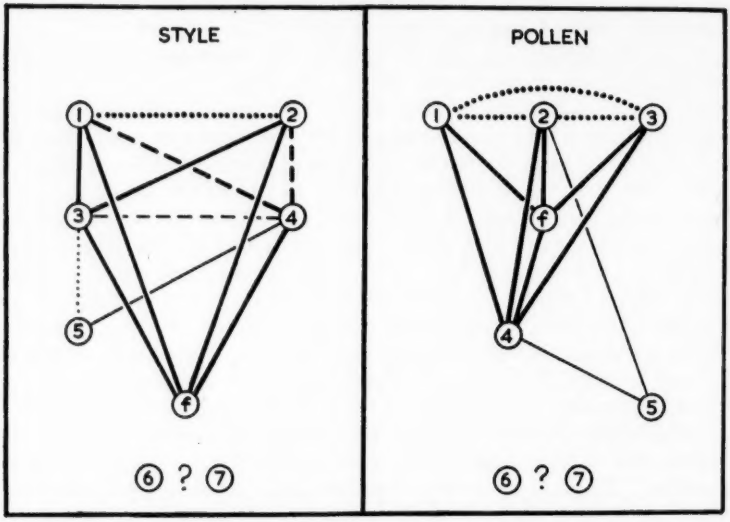


FIG. 9.—The **S**-alleles arranged spatially in accordance with their dominance relations.
Entire lines : dominance of upper allele over lower.
Broken lines : incomplete dominance of upper over lower.
(In the case of alleles **3** and **4** in the style, both alleles are slightly weakened.)
Dotted lines : independence of alleles (no dominance).
Thinner lines and smaller dots : evidence meagre.

Alleles **6** and **7** : These are placed at the bottom with a query because their activity was not proven in any genotype but they are not self-fertility alleles. They are presumed to be low in the dominance series.

4. DISCUSSION

In view of the rather novel interactions revealed between the **S**-alleles it is important to establish confidence in the experimental technique.

One hundred and fifty self-sterile plants were tested in 593 intra-family pollinations involving five or six flowers each, and many repeated. Only four plants did not fall into expected groups. One

of these was probably a male-sterile self-fertile as previously experienced. The other three could only be explained by contamination.

In the inter-family tests twenty-one plants were tested as females against a maximum of thirty-eight and a minimum of twenty-two plants, involving 605 pollinations (many repeated). No inexplicable results were obtained.

Examination of fig. 9 shows an overall similarity in the dominance relations in pollen and style but important differences in detail. In both, the alleles can be arranged in a linear order from top to bottom so that when dominance occurs it is usually of upper over lower. Independence of alleles may occur when they are at similar levels on this linear scale. Alleles which are independent in pollen can show dominance in style and *vice versa*.

The biggest difference between pollen and style is that the linear order of alleles **f** and **4** is reversed though there are other quantitative changes especially of **3** relative to **4** without involving a reversal of the positions of the alleles.

When multiple allelic incompatibility with dominance was first discovered (Gerstel, 1950; Hughes and Babcock, 1950) there was an apparent contradiction. It might have been expected that the existence of a large number of mutually exclusive specific S-alleles indicated multi-dimensional qualitative differences between them. A dominance series indicates a uni-dimensional quantitative difference. It was this contradiction which had led Lewis (1944) to conclude that such an incompatibility system did not exist. The present data throw some light on this contradiction. If we take the interactions in the pollen the alleles can be arranged in a linear order of dominance, and similarly, but not so consistently, in the style. But the two linear orders do not correspond. We can thus regard the overall relationship of the alleles as multi-dimensional, the dominance relationship representing the projection of these dimensions on to a line transept. We have only to assume the dominance in pollen and style to be represented by two unparallel lines through this multi-dimensional distribution to explain the change in dominance relationships. The shift of the dominance projection line from pollen to style would be due to the change of environment. If it were possible to measure the alleles in some other attribute in which they might vary, say rate of pollen tube growth, mutation rate, temperature at which destroyed and so on, the linear orders would be expected to vary more widely than does dominance between pollen and style.

5. COMPARATIVE GENETICS

It is desirable to compare the relationships of the alleles in *Iberis* with those in the few other known instances of sporophytic control of the pollen with multiple alleles.

In *Cosmos* (1954) Crowe has also found dominance and independence

in pollen and style with incomplete correlation between the behaviour in the two sexes.

In both *Crepis* and *Parthenium* the authors imply that the S-alleles are independent in style and sometimes show dominance in the pollen. Their own data contradict this however, because the results can only be explained by assuming some dominance in the style also. (In *Parthenium* **R4** is recessive to **R1**, **R2** and **R3**. In *Crepis* **S1** is recessive to **S2**, **S3** and **S4**). In any case each study of this phenomenon has involved only a small sample of alleles in each species. In my own experience my first study of *Iberis* showed almost complete correlation between dominance relations in the two sexes, the one exception

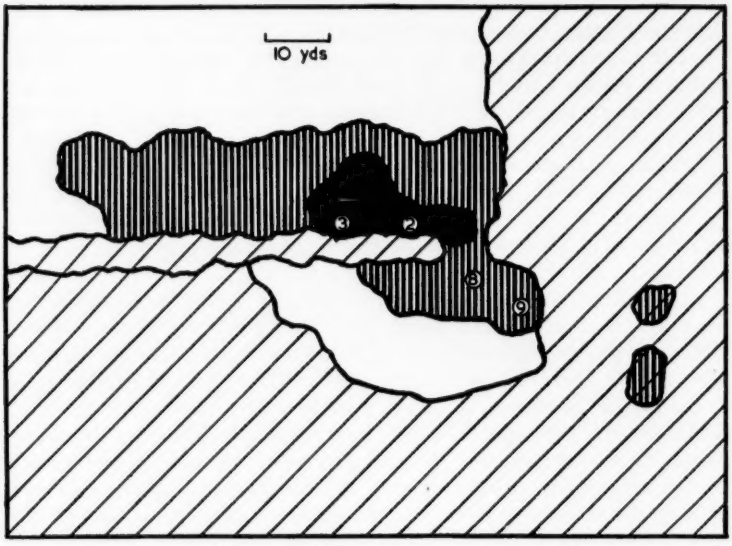


FIG. 10.—Map of the Royston Heath, Herts., population of *Iberis amara*.
Black area : main concentration.
Vertical shading : remainder of population.
Diagonal shading : woodland and hedgerow.
Numbered circles : sites of analysed seed samples.

being based on slender evidence. It is obviously dangerous to generalise from small samples when sporophytic control in style and pollen presents so many possibilities for variation. There is probably no real contradiction between the results so far obtained from *Parthenium*, *Crepis*, *Cosmos* in the Compositae and *Iberis* in the Cruciferae even though from the presence of *Iberis* in a totally different family one might have anticipated important differences.

It would seem likely that the simultaneous existence of dominance and independence between S-alleles, in both style and pollen, is a necessary accompaniment of incompatibility systems which combine multiple alleles with sporophytic control of style and pollen.

6. CROSS-INCOMPATIBILITY IN A NATURAL POPULATION

At the western margin of Royston Heath, Hertfordshire, there is a natural population of *Iberis amara*. According to Pryor's *Flora of Hertfordshire* there was in 1840 a population of this species " In and about a plantation adjoining west part of Royston Heath ". It appears that the same population has been in existence for over a century, for Pryor's description agrees with the present site. At the present time the population consists of thousands of individuals growing as annuals. It is reasonable to suppose therefore that an equilibrium has been achieved. Fig. 10 represents a plan of the area.

Samples of seed were taken from three immediately neighbouring plants from each of eleven sites throughout the area. Seed from only four of these sites produced enough adult plants for these experiments.

Two suitable tester plants from each " family " * were used as females for testing the compatibility of pollen from different sources, within the same family, between families from the same site, and between sites. Each family is represented by the site number with the plant number of the seed parent as index.

The test pollinations were as follows (each cross represents two tester plants and as many pollen donors as are members of the family).

TABLE 7

Within families	Within sites	Between sites
2^2	$3^2 \times 3^1$	$2^2 \times 3^1$
3^1	$3^3 \times 3^1$	$2^2 \times 9^2$
3^2	$3^3 \times 3^2$	$3^1 \times 9^2$
3^3	$8^3 \times 8^3$	$8^3 \times 2^2$
8^2	$8^3 \times 8^2$	$8^3 \times 3^1$
8^3	$9^2 \times 9^3$	...
9^2	$9^3 \times 9^2$	...
9^3	...	...

All pollinations were recorded as compatible or incompatible according to their stigma reaction but, as a check, the same crosses were scored later according to fruit setting. There were few cases of conflict between the two scorings. The general conclusions were the same with both scoring methods.

Out of 52 plants grown, 5 were discarded as self-fertile.

Of the pollinations within families 27 out of 82 (38 per cent.) were incompatible. In biparental families the average cross-incompatibility to be expected theoretically is 50 per cent. This is borne out by the earlier experiments of this paper. If we consider the two maternal alleles, one or other of which will be present in every plant even after open pollination, and assume that one of them is active

* By " family " is here meant the progeny of a single open-pollinated seed parent which will probably have several pollen parents.

in all the progeny bearing it, cross-incompatibility due to the maternal alleles alone would be 25 per cent. If both alleles were active, but in only half the plants carrying them, the cross-incompatibility would be down to 12·5 per cent. At the other extreme, if all alleles were active the maternally derived cross-incompatibility would be 50 per cent. It is probable that 33 per cent. cross-incompatibility comprises an appreciable amount of cross-incompatibility between paternal alleles, either through a common pollen parent or through pollen parents with common alleles.

Out of 71 pollinations between families with a common site 8 (11 per cent.) were cross-incompatible, and out of 67 between sites only 3 (4·8 per cent.) were cross-incompatible.

The above figures are based on all pollinations. Since so much depends on whether one or both of the alleles of any tester plant are active, and on the identity of the active allele, a more exact comparison is made if we confine ourselves to pollinations made on to a common tester plant.

The first two rows have data selected from tester plants common to the columns being compared. Unfortunately very few test plants were common to " within sites " and " between sites ". However, the results are the same for each row.

TABLE 8

	1. Within families		2. Within sites		3. Between sites	
	No. pollinations	Per cent. incomp.	No. pollinations	Per cent. incomp.	No. pollinations	Per cent. incomp.
Comparison 1-2	35	34	71	11	...	...
Comparison 1-3	30	33	...	...	67	4·5
All pollinations	82	33	71	11	67	4·5

Now if all alleles have an even chance of being active (penetrance 50 per cent.) and all individuals are heterozygous, the frequency of random pairs of plants with a common S-allele will be four times the frequency of cross-incompatibility. Furthermore, according to the argument of an earlier paper (Bateman, 1947*a*), if the frequency of random pairs with a common allele is n the total number of S-alleles is $4\ n$. If we apply these arguments to the data on cross-incompatibility between sites (4·5 per cent.) the proportion of random pairs with a common allele is 18 per cent. and the total number of S-alleles will be $4/0{\cdot}18 = 22$.

But we know that there is great variation in penetrance (even if the mean is 50 per cent.) according to the position of the allele in the dominance series. Furthermore we have evidence from the earlier experiments that independence is frequent, which would raise

the mean penetrance above 50 per cent. Again we would expect on theoretical grounds considerable variation in gene frequency (Bateman, 1952) for the more recessive alleles would be more frequent than the more dominant ones. Allowance for any one of these sources of error would increase the estimated number of alleles. We can safely assume therefore that 22 is an under-estimate of the number of S-alleles in this natural population of *Iberis amara*.

Further conclusions as to the structure of the population can be drawn from a comparison of the within-sites and between-sites pollinations. The greater cross-incompatibility within-sites demonstrates that even within a population extending about 30 yards in length (the distance between sites 9 and 3) there is no panmixis, but on the contrary a correlation between probability of inter-crossing (proximity) and relationships (as given by number of alleles in common).

It should be stated that the χ_2 for heterogeneity between the within-sites and between-sites pollinations is only 2·17 for one degree of freedom. This is not quite significant at the 5 per cent. level ($P = 0{\cdot}05$-$0{\cdot}10$) even after allowing that we can halve the probability given in the table since we are only expecting deviation in one direction. But the data on which they are based are very consistent and the conclusions are in any case to be expected from the evidence on the behaviour of pollinating insects presented in an earlier paper (Bateman, 1947*b*).

7. SUMMARY

1. A study has been made of self-incompatibility in three samples of the Cruciferous species, *Iberis amara*.

2. Incompatible pollen fails to penetrate the stigma, but compatible pollen promotes a flushing of the stigma within 24 hours of pollination. This coloration has been used as the standard test of compatibility.

Garden Races

3. (*a*) The two samples demonstrated an incompatibility system (hitherto observed only in the Compositae) combining sporophytic control of both pollen and style with a multiple series of alleles.

(*b*) The two alleles of a heterozygote may act independently or one may be dominant over the other. With certain pairs of alleles, one of the pair is weakened though still active.

(*c*) Dominance and independence are found in both pollen and style. The relationship of a given pair of alleles need not however be the same in both.

(*d*) The alleles can be arranged in a linear order of dominance, independent alleles being at the same position. The same alleles form a partially different series in pollen and style.

(*e*) Self-fertility is fairly common. In the first sample it was due to a recessive gene independent of the S locus. In the second

sample the self-fertility gene was a mutant at the S locus, and was recessive to some incompatibility alleles but dominant to others, at least in the pollen.

(*f*) Five parent plants from one of the samples shared six alleles. Six parents from the second sample shared seven or eight alleles.

Wild Race

4. (*a*) The degree of cross-incompatibility between unrelated plants indicated a large number of alleles, probably greater than 22.

(*b*) The cross-incompatibility between progenies of neighbouring plants approached that within progenies. Thus neighbouring plants are more closely related than a random sample from the population. *I.e.* there is no panmixis, even in a population extending over as little as 30 yards.

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GENE-ENVIRONMENT INTERACTION :

A RELATIONSHIP BETWEEN DOMINANCE, HETEROSIS, PHENOTYPIC STABILITY AND VARIABILITY

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1. GENE ACTION

DARLINGTON and Mather (1949) have classified gene action and interaction in four types :—direct, successive, cooperative and competitive. It is essential for the present study to add duplicate or parallel gene action, in which a particular phenotype is the outcome of two or more genes or alleles working towards the same end but by slightly different synthetic routes.

Enough examples of duplicate major genes are known in higher plants and animals and in micro-organisms to show that they are both widespread and frequent. Duplicate gene action implies that parallel, and therefore alternative, pathways are available between the successive steps from gene to character. Such alternative pathways may be determined either by allelomorphs or by different loci. Which pathway is in operation under a particular set of conditions will depend upon which allele or gene is dominant or epistatic under these conditions. Each gene will have its optimum set of conditions which comprise three components : (*a*) Genetic background (genic environment) ; (*b*) Stage of development (cytoplasmic environment), and (*c*) External conditions (external environment).

The present study is concerned with the effects of the cytoplasmic and external environment on average dominance as it affects a polygenic character and how this in turn affects the stability in external form of an organism.

2. THEORETICAL PRINCIPLES

(*a*) *Homozygotes*

The present attempt to analyse theoretically interactions arising between two pure lines, their F_1 and two different environments follows the more manageable analysis of the possible interactions with two genetically different populations in two environments made by Haldane (1947). But to keep the analysis within bounds only some of the extreme possibilities have been selected.

Let us consider a pair of alleles : A_1, A_2 affecting a measurable character such as height or flower number in plants or wing length in *Drosophila*. Let us assume that both alleles are positively promoting the expression of the character and that in some environments A_2

produces twice the effect of **A1**. We can ascribe character values to individuals such that **A1A1** = 1 and **A2A2** = 2. Furthermore let us assume two environments L.E. and H.E. Consider for simplicity the two extreme environmental effects which might be manifested according to the genotype observed : (1) L.E. and H.E. have the same effect and (2) H.E. produces a phenotype twice as large as that produced by L.E. All intermediate interactions, which are probably the most common, can be ignored without any serious effect on the general conclusions.

When reduced to these artificial but manageable proportions there are four basic situations between the two homozygotes and the two environments (table 1).

TABLE 1

Basic extreme situations for two homozygotes in two environments. The environmental difference is assumed to have either no effect or a two-fold effect

I

	A1.1	A2.2
L.E.	1	2
H.E.	2	4

II

	A1.1	A2.2
L.E.	1	1
H.E.	1	2

III

	A1.1	A2.2
L.E.	1	2
H.E.	2	2

IV

	A1.1	A2.2
L.E.	1	1
H.E.	1	1

In situation I, H.E. has twice the effect of L.E. with both homozygotes ; in II the difference is shown only with **A2.2** ; in III with **A1.1** and in IV with neither of the homozygotes. The effect of the genotype is apparent in both environments in situation I, in only one environment in situation II and III, and not observable at all in situation IV.

We can for convenience call L.E. the *low-expression* and H.E. the *high-expression environment* : and since the extent of the environmental effect on the phenotype is one of the main aspects to be considered we must introduce the concept of *phenotypic stability*. This can be defined as the ability of an individual or population to produce a certain narrow range of phenotypes in different environments. It is similar to the concept of *homeostasis* (*cf.* Dobzhansky and Wallace, 1953). A convenient measure of phenotypic stability is the *stability factor*, which can be expressed as :—S.F. $= \frac{\bar{x}\ \text{H.E.}}{\bar{x}\ \text{L.E.}}$ where $\bar{x}$ is the

mean character value. This factor can be applied to: (1) between similar parts of the same individual, (2) to means of similar parts in different samples, (3) means of populations. The maximum phenotypic stability is attained when S.F. = 1, for then the phenotype is unaffected by the environment; the greater S.F. deviates from unity the less stable is the phenotype.

(*b*) *Heterozygotes—Dominance*

If there is no dominance of A_1 or A_2 then the heterozygote has an intermediate value between the two homozygotes in both L.E. and H.E., and we have in situation I the following relationships :—

Gen. / Env.	$A_{1.1}$	$A_{1.2}$	$A_{2.2}$
L.E.	1	1·5	2
H.E.	2	3	4
S.F.	2	2	2

In this example the heterozygote has the same stability factor as the two homozygotes. We are now able to consider the heterozygotes in the four basic homozygotic situations.

If we exclude superdominance, there are nine possible relationships of the heterozygote to the homozygotes: these are shown for situation I in table 2. They have been arranged in columns and rows according to the dominance relationships: the stability factors for heterozygotes given at the base and right side are means for each of the three basic dominance variants which are given at the top and left-hand side of the table.

It should be noted that the stability factor of both parents is 2·0 in situation I and therefore any hybrid with an S.F. below 2 is more stable than its parents and one with S.F. greater than 2 is more variable. The stability factors for the heterozygotes in the nine variants (*a-i*) in the four basic situations (I-IV) are summarised in table 3. The S.F.'s for situation I are taken from table 2 and those for the other situations have been derived in a similar way to those of situation I.

By comparing the hybrid S.F.'s with the mean parental values in table 3 it will be seen that certain generalisations about the interactions can be made.

1. A heterozygote which is more stable than the parental homozygotes is obtained when the gene which is dominant in a particular environment has an effect opposite to that of the environment, *e.g.*

small>large in the high expression environment and large>small in low expression environment.

2. A less stable heterozygote is obtained when the gene which is dominant in a particular environment has an effect which is similar to that of the environment, *e.g.* small>large in the low expression environment and large>small in the high expression environment.

TABLE 2

The nine possible dominance relationships with the two environments L.E. and H.E. in situation I, in which both homozygotes are affected similarly by the environments. The stability factors (S.F.) are means of the three variants in each column and row

DOMINANCE ——→	$A_1 \ldots A_2$ in H.E.	$A_1 > A_2$ in H.E.	$A_2 > A_1$ in H.E.
	(*a*) $A_{1.1}$ $A_{1.2}$ $A_{2.2}$	(*b*) $A_{1.1}$ $A_{1.2}$ $A_{2.2}$	(*c*) $A_{1.1}$ $A_{1.2}$ $A_{2.2}$
$A_1 \ldots A_2$ in L.E. L.E.	1 1·5 2	1 1·5 2	1 1·5 2 — 2·0
H.E.	2 3 4	2 2 4	2 4 4
	(*d*)	(*e*)	(*f*)
$A_1 > A_2$ in L.E. L.E.	1 1 2	1 1 2	1 1 2 — 3·0
H.E.	2 3 4	2 2 4	2 4 4
	(*g*)	(*h*)	(*i*)
$A_2 > A_1$ in L.E. L.E.	1 2 2	1 2 2	1 2 2 — 1·5
H.E.	2 3 4	2 2 4	2 4 4
	2·0	1·3	2·6 ←——— S.F.

Note that to get a more stable hybrid the gene which is dominant in a particular environment must have an effect opposite to that of the environment. *E.g.* when small>large in high expression environment S.F. = 1·3 : and when large>small in low expression environment S.F. = 1·5. (All S.F.'s for homozygotes = 2·0.)

$A_1 \ldots A_2$ = no dominance. $A_1 > A_2 = A_1$ = dominant to A_2.

3. In general, complete lack of dominance under all conditions tends to have no differential effect on the stability of the heterozygote as compared with the homozygotes.

(c) *Superdominance—Heterosis*

It is now left to consider single-locus heterosis or *superdominance*. We must distinguish positive heterosis in which the heterozygotic

phenotypic value is greater than either the homozygotic parental values, and negative heterosis when the heterozygotic value is less than that of the homozygotes. Positive heterosis is exhibited as :—

$A_{1.1}$	$A_{1.2}$	$A_{2.2}$
1	3	2

in L.E. and as

$A_{1.1}$	$A_{1.2}$	$A_{2.2}$
2	6	4

in H.E. The corresponding heterozygotic values with negative heterosis are 0·75 in L.E. and 1·5 in H.E. The effect of positive and negative heterosis on the stability factors is given in table 4.

TABLE 3

The stability factors obtained in the four basic situations. There are six stability factors for each situation obtained in the same way as shown in table 2. Double squares denote an S.F. lower than the parental mean S.F.

				I	II	III	IV	
				2·0	1·5	1·3	1·0	$A_1..A_2$ in L.E.
				3·0	1·5	2·0	1·0	$A_1>A_2$ in L.E.
				1·5	1·5	1·0	1·0	$A_2>A_1$ in L.E.
I	2·0	1·3	2·6	2·0				
II	1·5	1·0	2·0		1·5			
III	1·3	1·3	1·3			1·5		
IV	1·0	1·0	1·0				1·0	
	$A_1..A_2$ in H.E.	$A_1>A_2$ in H.E.	$A_2>A_1$ in H.E.	Mean parental S.F.				

The effect is similar but more extreme to that of dominance, and the general conclusions 1 and 2 derived from dominance relationship apply also to *superdominance*.

Application.—The theoretical interactions based on a pair of alleles is only the model for the types of interaction with a polygenic system. A similar model has proved its worth in the analysis of other genetic characteristics of polygenic systems by Fisher and Mather. The one gene model can be transferred to a polygenic system by using such terms as " average dominance " or potence instead of dominance.

Examples in the literature of changes in dominance of single major genes by environment are not common—this is partly because of the

lack of suitable experiments to reveal such changes but the examples which are known are of the extreme change as shown in situation I (*f*) in table 2, *i.e.* complete reversal of dominance. Examples are Honing's (1928) light requiring seeds of *Nicotiana* in which light requirement is dominant in young seeds but recessive in old; and in *Drosophila*, infrabar eye is dominant to bar eye at 17° C. but recessive at 25° C. (Hersh, 1934).

Since complete reversal of dominance has been found it can be assumed that the less drastic changes, which are the basis of the theoretical analysis, occur.

TABLE 4

The effect of positive and negative heterosis on the stability factors of the heterozygotes. Note that increased stability (when S.F. = 1·0) is obtained when the direction of the heterotic effect is going against the effect of environment

		A1.1	A1.2	A2.2		A1.1	A1.2	A2.2
Positive heterosis	L.E.	1	3	2	L.E.	1	1·5	2
	H.E.	2	3	4	H.E.	2	6	4
	S.F.		1		S.F.		4·0	
		A1.1	A1.2	A2.2		A1.1	A1.2	A2.2
Negative heterosis	L.E.	1	0·75	2	L.E.	1	1·5	2
	H.E.	2	3	4	H.E.	2	1·5	2
	S.F.		4·0		S.F.		1·0	

3. MATERIAL

(*a*) *Character-varieties*

The polygenic character used for the experiment is the number of flowers per inflorescence in the cultivated tomato, *Lycopersicon esculentum*. It was chosen because environmental effects on the character expression can be easily controlled. All the material came from two varieties, Kondine Red, which of a large number of varieties had the highest number of flowers and Vetomold which had the lowest number. Kondine Red (K.R. in the text) is an old English greenhouse tomato; Vetomold (V.M.) is a disease-resistant variety which had *Lycopersicon pimpinellifolium* in its ancestry, but which shows only slight evidence of its interspecific origin.

(*b*) *Inbreeding*

Although English tomato varieties are mainly self-pollinated, K.R. and V.M. were found to be heterozygous for some genes controlling flower numbers because a slight response to selection for low and high number was obtained. Inbreeding by self-pollination was practised until selection failed to give a response: this took three generations.

4. METHODS

Seeds were sown in John Innes No. 1 compost and germinated at a constant temperature of 30±1° C. in a glass-sided incubator. Seedlings were transferred to pots of No. 2 compost and at the same time placed in different temperatures. The seedlings were kept in these environments until the initials of the first inflorescence were visible: during this period temperature has a profound effect on size of the first two or three inflorescences. The critical period for the different inflorescences and details of the effects of environment have been described by Lewis (1953).

For important experiments the environments during the critical stage were accurately maintained in specially controlled environment rooms in which the light is entirely artificial from 5 ft. 80 watt daylight fluorescent tubes giving an intensity of 1500 ft. candles at plant height and a day length of 14 hours. The temperature was controlled to within 0·5°: the high expression environment was 13° and the low expression environment was 25°.

Less critical experiments had to be done in environments which were to some extent under the influence of the weather. These were a cold frame and warm greenhouse: in some years it was easy to maintain a satisfactory difference in temperature between them but in others, when high outdoor temperatures prevailed, this was not possible. In all experiments inbred material as standards were grown as a measure of comparison between years. After the period in the critical environments the plants were transplanted to the open ground out-of-doors, where observations, selections and pollinations were made.

Both in the critical environments and in the later outdoor plots, the plants were arranged in randomised blocks.

5. PLAN OF THE EXPERIMENT

The experiment consisted of two parts:—

1. A comparison between parents F_1, F_2, 20 F_3 families and backcross generations. The 20 F_3 families were derived by selfing F_2 plants taken at random in 1951. All these were raised in 1952 in eight randomised blocks: each block contained 28 plants of each family divided between 14 plots. The randomised order of the plants in the critical environments was maintained when planting out in the open ground. Four blocks were raised at 13° C. and four at 25° C. The four blocks at each temperature were sown at different times, following each other through the rooms: the 13° treatments were sown at five-weekly intervals and the 25° at four-weekly intervals, and planted out at corresponding periods. This meant that the environments in the rooms were the same for all replicate blocks but the later environments after planting were different between blocks and the consequences of this will be apparent later.

The critical environment in the controlled rooms during the second to the fifth week after sowing affects the size of the first two or three inflorescences but the fourth and fifth inflorescence will be laid down when the plants are outside. Thus the 1st and 2nd inflorescence will have the same treatment in replicate blocks while the 4th and 5th will have different treatments between the blocks owing to the natural changing temperature and light conditions with the advance of the season.

The comparisons that can be made due to environmental and developmental influences are shown in fig. 1.

The comparison L.E. : H.E. which is made *between* individuals, can be calculated only between means of families. The comparisons L.E. : E_X and H.E. : E_X, which are made *within* individuals, can be calculated either on individual plants or on the means of the families.

2. The second part of the experiment is the continued selection from the F_2

family for high and low numbers under high expression and low expression environments. The two environments for these experiments were provided by the cold frame and heated greenhouse. After some generations of selection, hybrids were made between different lines as a comparison with the original F_1 and parents.

6. RESULTS OF EXPERIMENTS WITH P_1, P_2 AND DERIVATIVE GENERATIONS

Inflorescence I

The means of the first inflorescence with their standard errors of the different families in the four blocks of each treatment are given in table 5. The standard errors have been calculated not from between

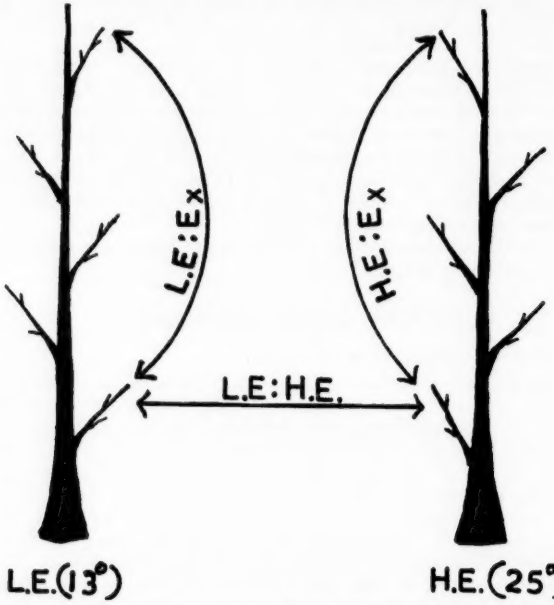

FIG. 1.—Shows the comparisons which can be made between environmental effects. Each plant is allowed to develop five inflorescences, the lower two are formed under the influence of the controlled environments, L.E. and H.E.; the upper two are formed under the influence of the uncontrolled natural environment, E_x.

plots but from between plants by ignoring the subdivision into plots because of the extremely small difference between them.

In general the differences between the blocks are small and not significant, but three significant deviations are found which have technical explanations. Block IV in the H.E. has an abnormally low value for the P_1. This was due to the difficulty of counting the flowers in these plants because of their extreme lateness and the bad weather which prevented the younger buds from developing. The same difficulty does not occur with the other

families in this block because the smaller inflorescences finish developing earlier.

Block IV in the L.E. suffers from an abnormally high within family variance in certain families including the F_1 which is of major importance in the subsequent analysis. This high variance is reflected in the standard errors given in table 5. Block II in the L.E. has significantly higher mean values for all families. This anomaly was due to a breakdown in temperature control during one day when the temperature rose to 31° C. The result of this rise in temperature was to prevent the formation of the true first inflorescence so that the first on

TABLE 5

Means of flower numbers in the first inflorescence for parents and derivative generations

(Abnormal blocks heavily outlined)

		Block I	Block II	Block III	Block IV
H.E.	P_1	20·68±1·25	18·61±1·13	18·28±1·09	15·29±0·62
	B_1	14·93±1·17	14·61±1·03	12·93±0·83	14·89±0·91
	F_1	13·89±0·89	13·07±0·61	11·43±0·76	12·89±0·85
	F_2	12·28±0·83	13·89±0·81	13·21±0·94	12·19±0·63
	F_3	12·60±0·56	12·46±0·54	11·29±0·49	11·82±0·51
	B_2	13·39±0·56	12·43±0·55	11·50±0·74	13·14±0·61
	P_2	12·28±0·75	12·75±0·63	11·46±0·65	13·96±0·70
L.E.	P_1	12·43±0·93	16·77±2·81	11·93±0·73	12·85±0·94
	B_1	11·14±0·75	17·82±3·28	11·58±0·76	10·68±0·63
	F_1	9·96±0·34	14·46±0·76	10·67±0·28	11·61±0·94
	F_2	8·46±0·64	12·81±1·60	9·70±0·95	10·89±0·93
	F_3	8·61±0·39	11·99±0·58	9·21±0·42	8·92±0·40
	B_2	7·36±0·39	9·11±0·65	9·29±0·40	8·39±0·46
	P_2	6·96±0·41	8·00±0·83	6·75±0·33	7·61±0·36

the plant was produced in the normal position of inflorescence II, *i.e.* above the 12th instead of the 9th leaf. The mean number of flowers in all inflorescences in this block were abnormal due to this displacement.

In all the subsequent analyses on the first inflorescences these three abnormal blocks have been discarded, thus making three blocks in the H.E. and two in the L.E. treatments. It is not legitimate to combine the data of the later inflorescence from different blocks because these inflorescences were laid down under the outdoor conditions. The combined data for inflorescence I are given in fig. 2.

It can be seen that the temperature treatments have an effect on all families but more so on the parents than on the F_1 and F_2 generations. The dominance of the genes is also changed by the temperature. The small size of the P_2 being almost completely dominant in the low temperature as shown by the great disparity between the F_1 mean and the Mid parent (M). At the high temperature dominance is absent. This is therefore an example of the theoretical situation I*b* (*cf.* table 2).

Attempts have been made to find the best scale for the data in order to remove interactions (*cf.* Mather, 1949) and hence facilitate the analysis. This has not proved satisfactory because of the different interactions with the different environments. The untransformed data of the L.E. fit the expected values in the scaling tests, while the H.E. shows some significant deviations which are not removed by logarithmic scaling. Thus it is clear that no one scale will fit all the data as was found in *Nicotiana* by Mather and Vines (1952) and in *Lycopersicon* by Powers (1950). Therefore all the analysis has been on untransformed figures.

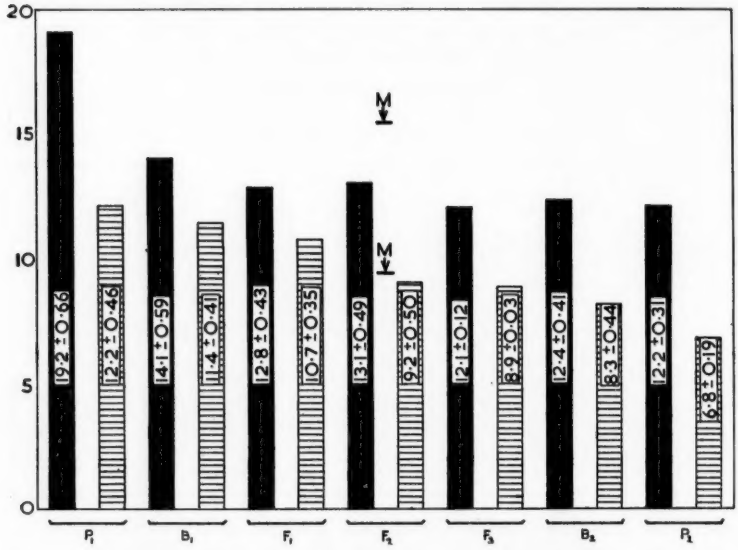

FIG. 2.—Means and standard errors for the two parents and derivative generations raised in different environments :

H.E. (13°). L.E. (25°). M = mid parent value.

Note the almost complete dominance of P_2 in the H.E. and the intermediate value of the F_1 in the L.E.

To show the reaction of the different families to the two environments the stability factors S.F. $= \left(\frac{\text{mean in H.E.}}{\text{mean in L.E.}}\right)$ have been plotted in fig. 3. The F_1 is the least affected by the environments, the two pure line parents the most affected while the other families have intermediate values. It is possible to calculate expected values for these families by assuming that heterozygotes will have the F_1 value and the homozygotes will have a value similar to their parents and that there are no interactions.

Thus the expected values are calculated as follows :—

$$B_1 = \frac{P_1+F_1}{2} \quad F_2 = \frac{P_1+P_2+2F_1}{4} \quad F_3 = \frac{P_1+P_2+2F_1+4F_2}{8}$$

$$B_2 = \frac{P_2+F_1}{2}$$

The values compared with observed figures show reasonable agreement as follows

	B_1	F_2	F_3	B_2
Observed . .	1·23	1·42	1·35	1·49
Expected . .	1·38	1·43	1·42	1·49

The B_1 differs by 0·15 from the expected, this is another confirmation of the dominance of the genes from the P_2 as shown in fig. 2.

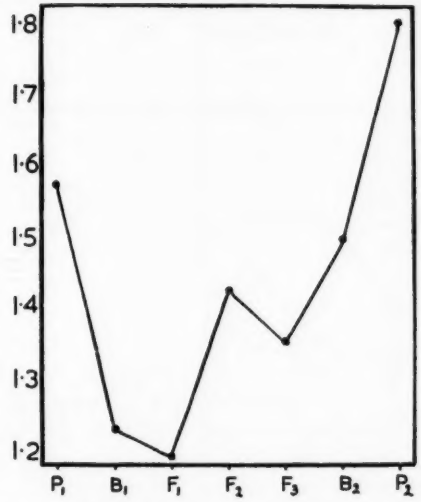

FIG. 3.—Stability factors $\left(\frac{\text{mean in H.E.}}{\text{mean in L.E.}}\right)$ for parents and derivative generations : note the greater stability of the F_1 generation.

Inflorescences I-V

Of the four randomised blocks only the plants in the first block could be scored for all five inflorescences. The means of the flower numbers for each inflorescence for the two parents, the F_1 and back-cross generations are plotted in fig. 4.

It is evident from this figure that the early temperature treatments (L.E. and H.E.) have effects on the first two inflorescences only.

Between the second and third inflorescences there is a switch over so that the treatment producing the highest number in the first and second inflorescences later gives the lowest numbers. There is also a tendency in all families and treatments to have larger inflorescences

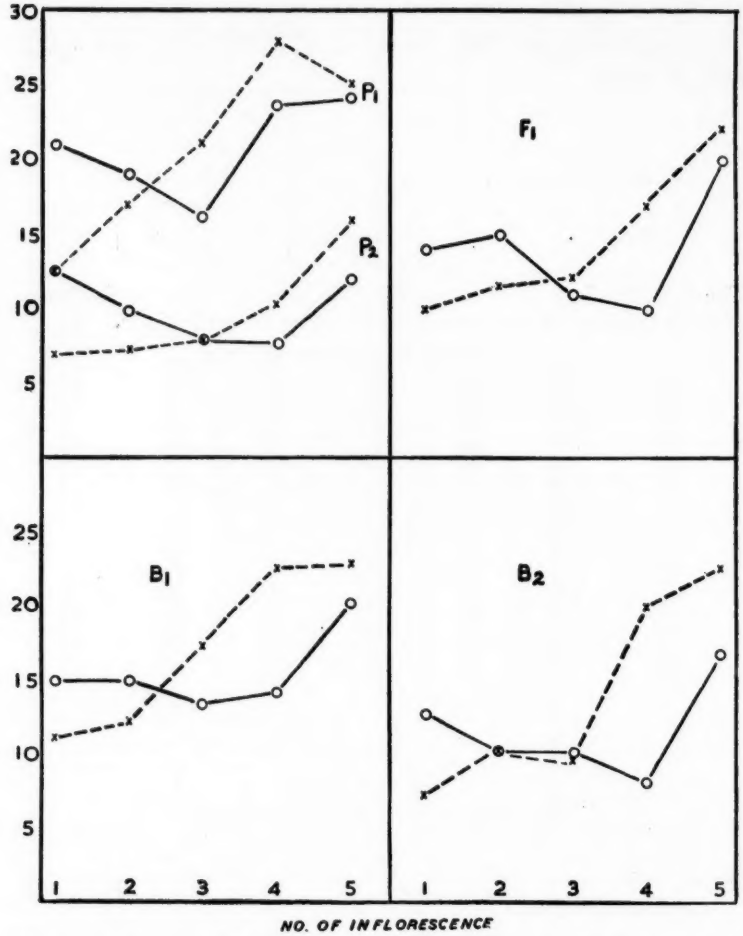

FIG. 4.—Mean number of flowers plotted for the five inflorescences in parents, F_1 and backcross generations (data from block I). Note that the effects of the two treatments switch over between the 2nd and 3rd inflorescence : ——— H.E., - - - - - L.E.

in the fifth position. Finally by comparing the parental curves with the F_1 it will be seen that the dominance is changed from the first to the fifth inflorescence. In the L.E. lack of dominance in the first is changed to a high degree of dominance of P_1 in the fifth, while in

the H.E. dominance of P_2 is completely reversed to dominance of P_1 in the fifth inflorescence.

We have seen that the different temperature treatments also changed the dominance as measured by inflorescence I. These dominance relationships will be discussed later.

7. SELECTED LINES

In 1949 selections were begun on F_2 families between the K.R. and V.M. pure lines. Single plants which were self-pollinated were

TABLE 6

Mean flower number of 1st inflorescence for selected F_6 lines and their hybrids in two environments—note the negative heterosis in hybrids 3 and 4 expressed only in the H.E.

	Parents		F_6 selections							
	K.R.	V.M.	* 1	2	* 3	4	* 5	* 6	* 7	* 8
H.E.	33·5	12·7	42·5	13·2	49·6	13·5	43·7	47·9	38·8	43·8
L.E.	9·7	6·3	11·1	6·4	12·3	7·0	12·7	11·7	11·3	11·9
	Hybrids		1		2		3		4	
H.E.			20·7		23·3		30·5		25·7	
L.E.			8·7		8·4		12·3		12·0	

		Stability factors			
S.F.	Parental mean .	2·9	2·9	3·6	3·9
	F_1. . . .	2·3	2·7	2·4	2·1

* High-selection lines.

selected for high and low flower number and in two different environments. These selections have progressed to the F_6 generation, and with the method of selfing adopted all the lines became fixed at either the fourth or fifth generation. The details of the results do not warrant publication here but the values obtained with the selected lines and the results of crosses between them are relevant to the subject of this report and are given in table 6.

It should be pointed out that these selected lines were not raised in controlled environment rooms as were the plants in the main experiment. They were raised in a hot greenhouse for the L.E. and in a cool frame for the H.E. The difference in effects of these two environments was greater than that between the corresponding treatments with the main experiment but a comparative check was provided by including the two pure line parents.

It is apparent from table 6 that all the high selections (1, 3, 5, 6, 7 and 8) have many more flowers in the H.E. than the highest parent ; while in the L.E. they have only slightly higher values. The two low selections (2 and 4) on the other hand have values which are the same as those of the low parent.

The two hybrids (3 and 4) between two high lines show that in the L.E. all the parents and the F_1's appear to be identical but in the H.E. the F_1's have values which are much lower than either of their parents. Thus although the lines must have different genes, these are only in operation in the H.E.

These two examples of negative heterosis shown only at the H.E. are another manifestation of the dominance of the small parent which is expressed only in this environment as shown in fig. 2. As with the main experiment the F_1 plants are less affected by the two environments than their parents. This is shown by the S.F. values. The theoretical model of this type of heterosis-environment interaction has been considered in table 4.

8. EFFECT OF UNCONTROLLED ENVIRONMENTAL DIFFERENCES

Despite the fact that the environment during the sensitive period for flower production was controlled, there were very considerable differences between individual plants within the pure lines and the F_1's which could not be due to genetic differences. They are due to " intangible environmental effects produced by developmental accidents or uncontrolled non-genetic variation with equal probability of affecting any member of a population " (Lerner, 1950). A measure of such effects can be obtained by the variability between plants within a homozygous line or between different parts on the same plant. In the present experiments the different inflorescences on a plant are formed under different experimental environments. Thus if we used the variance between inflorescences on the same plant as a measure, the effects we are looking for would be masked by the gross environmental effects. But differences between plants within any one treatment block will give a valid estimate of these intangible environmental effects.

As a measure of variability the standard deviation and coefficients of variation (c.v. $= \frac{\sqrt{v}}{\text{mean}} \times 100$) have been calculated for parental lines and F_1 generations. The coefficient of variation, as pointed out by Day and Fisher (1937), gives a valid comparison only when the standard deviation is proportional to the mean. In the present data the regression coefficients of the standard deviations on the means are only slightly above unity, and therefore their use is unlikely to lead to serious error.

9. DOMINANCE AND VARIABILITY

The combined data for the first inflorescence are summarised in table 7. The regression coefficient of standard deviation on the mean is 1·4. Both the standard deviations and the coefficients of variation

TABLE 7

Means, standard deviation and coefficients of variation for parents and F_1 in the two environments L.E. and H.E. Note lower F_1 variation only in the L.E.

	L.E. mean	Block I and III		H.E. mean	Block I, II and III	
		S.D.	C.V.		S.D.	C.V.
P_1	12·18	*4·403*	36·14	19·19	*6·006*	31·29
F_1	10·31	*1·607*	*15·59*	12·79	*3·990*	*31·19*
P_2	8·32	*1·907*	22·92	12·16	*3·562*	29·29

indicate that in the L.E. the F_1 is less variable than its parents while in the H.E. it has equal variability with its parents. In the L.E. the

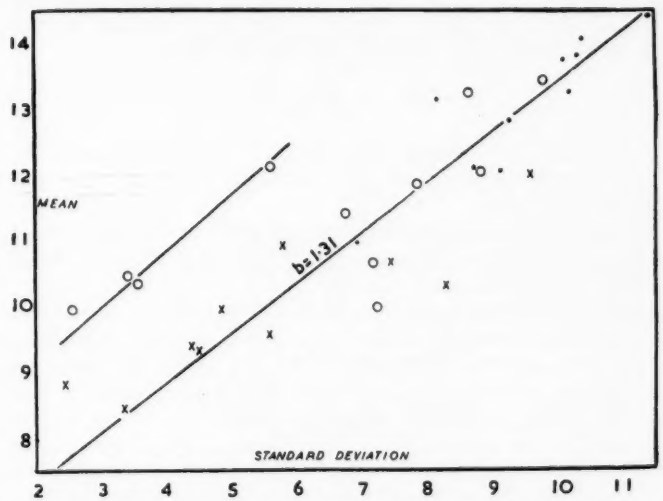

FIG. 5.—The regression of standard deviation, on the mean plotted for parents and F_1 for different treatments with different inflorescences. The regression line, $b = 1·31$, is based on parents only. Note the bad fit of the four F_1 generations which are indicated by a line estimated from the points: . = P_1, x = P_2, o = F_1, figures transformed to $10 \times \log^{10}$.

mean of the F_1 is intermediate between the parents thus showing no dominance, but in the H.E. the small parent (P_2) is almost completely dominant.

To see whether this is a general relationship between dominance and variability the data from all inflorescences in block I have been assembled. The mean and standard deviations transformed into $10 \times \text{logarithm}^{10}$ together with the regression line ($b = 1{\cdot}3$) calculated on the parents only are plotted in fig. 5. It will be seen that the parental values fit reasonably well to the regression line, but that while some of the F_1 values fit the line others do not. Those which do not fit are less variable than their parental lines. This confirms and extends the data based only on inflorescence I (table 7).

The relationship between dominance and variability suggested in table 7 can be tested if we have valid measures of average dominance and of F_1 variability relative to the parents. A measure of average dominance has been devised by Wigan 1944 in the formula $p = \dfrac{P_1 - F_1}{P_1 - P_2}$ where P_1, F_1 and P_2 are mean values for the respective lines. If we exclude heterosis the value of p may have any value from zero, when P_1 is dominant, to unity, when P_2 is dominant (P_1 being the larger parent) : when there is no dominance, $p = 0{\cdot}5$. With positive heterosis p has a negative value, and with negative heterosis p is greater than unity.

As a measure of F_1 variability relative to its parents I propose to use the standard deviation or some appropriate function of it in a *relative variability* function which is obtained as follows :

$$\text{R.V.} = \frac{2F_1 f(v)}{f(v)(P_1 + P_2)}$$

A value of unity is obtained when the F_1 is as variable as the mean of the parents, and the lower the value the less variable is the F_1 relative to the parents.

The dominance values and the values of R.V. are plotted for the data from the first five inflorescences in both the L.E. and the H.E. from block I in fig. 6.

It is evident from this figure that the F_1 relative variability is at its lowest when the dominance value is $0{\cdot}5$, and that it rises as the dominance deviates on either side of the $0{\cdot}5$ value. For dominance values ranging from $0{\cdot}5$ to $1{\cdot}0$ there is a good agreement with a linear relationship. On the other side, from $0{\cdot}5$ to $0{\cdot}0$, the three points are too few to show any general relationship. Some of these points are more reliable than others, those which are obtained from the second inflorescence are of less value than the others because at this inflorescence there is a change over occurring from the early controlled environments to the later condition. If the point C_2, which is from the 2nd inflorescence, on the left-hand side of the $0{\cdot}5$ dominance value is discarded the points are not incompatible with a general relationship similar to that on the right side of $0{\cdot}5$.

The data in fig. 6 are from the same parents and F_1 in different environments and from different parts of the plant. The F_6 selected

lines and their F_1's (table 6) have been analysed in the same way and are in agreement, thus showing that the dominance-variability relationship holds not only with different conditions but with different genotypes.

The two selected lines, which when intercrossed, showed negative heterosis will be referred to later.

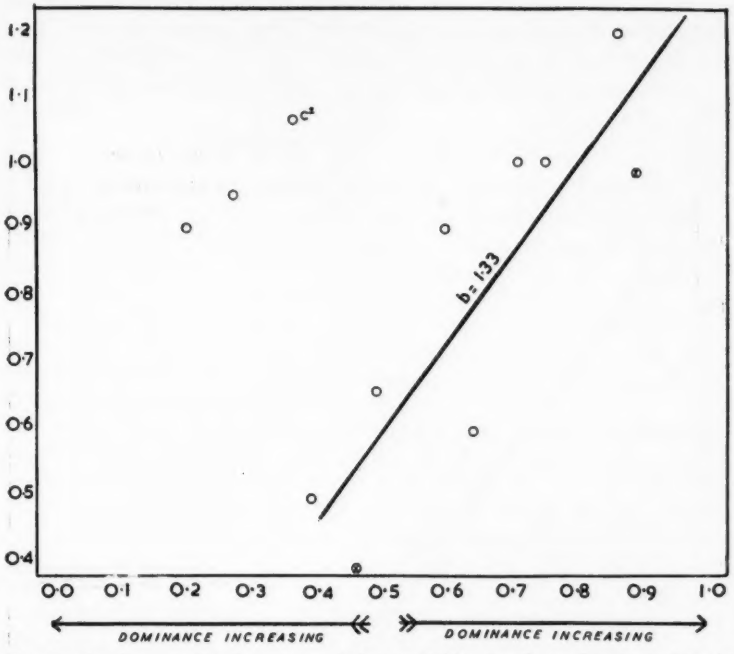

FIG. 6.—Dominance plotted against relative variability based on flower numbers in tomatoes. The o points represent data from different inflorescences and different treatments in block I. The ⊗ points represent the combined data from different treatments on the first inflorescence only. Note that with minimum dominance (0·5) there is a minimum value of the relative variability, and that as dominance increases on either side of the 0·5 value the relative variability increases.

10. DOMINANCE AND F_1 VARIABILITY IN THE PUBLISHED DATA

Much of the published data on polygenic characters does not include sufficient detail to be able to make a comparison between dominance and F_1 variability, but a search has revealed data in *Nicotiana rustica* given in table 10.1, p. 163 by Smith (1952) which can be analysed in the same way as the Tomato data. The *Nicotiana* data are from different genotypes, which make them particularly interesting as a comparison with the Tomato data. The characters analysed are plant height and leaf length in four parental lines and the six hybrids between them. Standard deviations have been calculated from the published variances, and with both height and

leaf length the correlation between the mean and standard deviation is so low that the graphical representation given in fig. 7 is made not on the coefficient of variation as with the Tomato data but on the standard deviation. Only F_1 families which do not show heterosis are included in this graph : the others will be considered later.

It is fortunate that the characters in *Nicotiana* tend to show dominance of the large parent, for they give a good relationship of the left side of the dominance value 0·5, thus complementing the

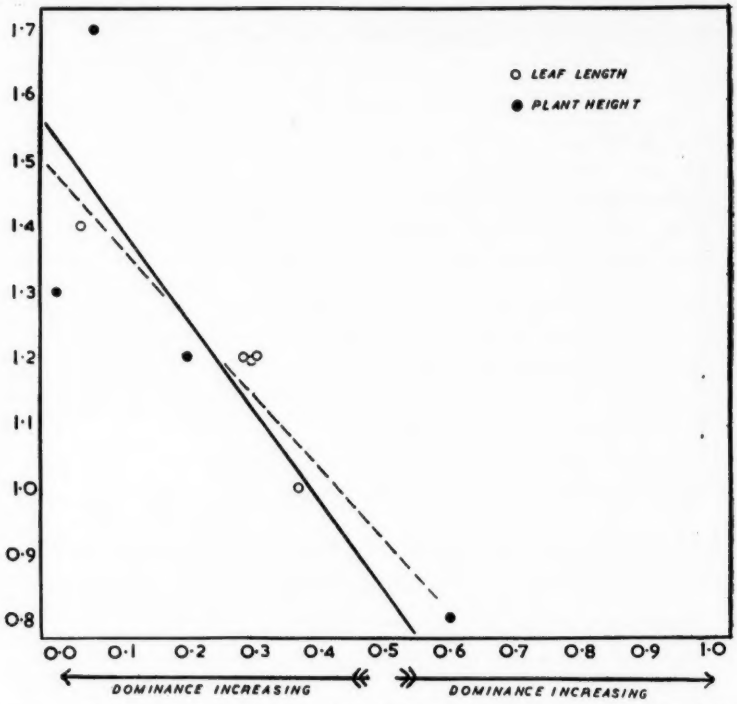

FIG. 7.—Dominance values plotted against relative variability based on *Nicotiana* hybrids (Smith, 1952). Note the low value of the relative variability with the minimum dominance.

Tomato graph. The probable straight line relationship for both these characters indicates that it may be of general occurrence.

In *Galeopsis* Hagberg (1952) has analysed the dry weights of many parental lines and their hybrids and has plotted the mean dry weights against the standard deviations in fig. 31, p. 225. All these hybrids either show complete dominance of the larger parent or positive heterosis. The graph shows that the F_1 families are as variable as the parents. This is in complete agreement since there is no lack of dominance in any of the hybrids.

In barley, Gustafsson (1946) has shown that plants which are heterozygous for a lethal gene are more variable than the homozygotes : this again is in complete accord since the normal allele is dominant.

All these examples are with inbreeding species, but an example from an outbreeding species is found in Mather's (1949) species cross between *Petunia axillaris* and *P. violacea*. The mean of the corolla length of the hybrid was intermediate between the two parents and the variability was much lower than the parents. This is again in agreement.

II. HETEROSIS AND F_1 VARIABILITY

Two of the Tomato hybrids between selected F_6 lines showed negative heterosis. Three of Smith's *Nicotiana* families showed positive heterosis : three of Hagberg's *Galeopsis* families also showed positive heterosis. In all these examples with heterosis the variability quotient is never below one and is generally slightly above one.

Therefore the three species examined all agree in showing lower F_1 variability only in the absence of dominance, and that heterosis either negative or positive like dominance results in an F_1 which is slightly more variable than the parents.

It is unfortunate that, although the majority of the work published on quantitative characters and heterosis is in naturally outbreeding organisms, the extensive data are not in a form fit for analysis on the dominance-variability relationship. For it would be extremely interesting to see whether the relationship holds with outbreeding organisms.

All the published results on the variability of the F_1 relative to their parents has not taken dominance into account. Many of the results give only the coefficients of variation without the means. Mather (1950) has shown that in the outbreeder *Primula sinensis* the variation in style length is lower in F_1's than in pure lines. Rasmusson (1951) and Robertson and Reeve (1952) found lower F_1 variabilities in *Drosophila melanogaster*. In four *Drosophila* species a lower F_1 variability for fertility has been found by Dobzhansky and Wallace (1953).

Dobzhansky and Wallace have related the reduced F_1 variability to the type of balance an organism has attained by its breeding system. They argue that an outbreeding organism will be more balanced physiologically in the heterozygous condition while an inbreeder will be more balanced as a homozygote. The increased F_1 variability of the inbreeding barley (Gustafsson, *loc. cit.*) and the decreased F_1 variability in outbreeders such as *Primula* and *Drosophila* are quoted to support this view. There is no doubt that the degree of heterozygosis which is optimum for fitness will be higher in an outbreeder than in an inbreeding organism. But the present results with Tomato and the quoted results from *Nicotiana* throw considerable doubt on

the conclusion that this difference in optimum heterozygosity is the cause of the different F_1 variabilities—if a difference there is between outbreeders and inbreeders.

12. THEORY OF ALTERNATIVE GENETIC PATHWAYS

The present approach to the problem of F_1 phenotypic stability and reduced variability is through gene action. In its simplest form we may assume a single biochemical change from a substrate (S) to a product (P). This may be performed by one gene **A**; a homozygote $\mathbf{A_1A_1}$ will have only one genetic pathway between S and P while a heterozygote $\mathbf{A_1A_2}$ may have one or two pathways according to the action of the alleles. This will also apply to different loci A and B having duplicate effects. The essential point is that the actions

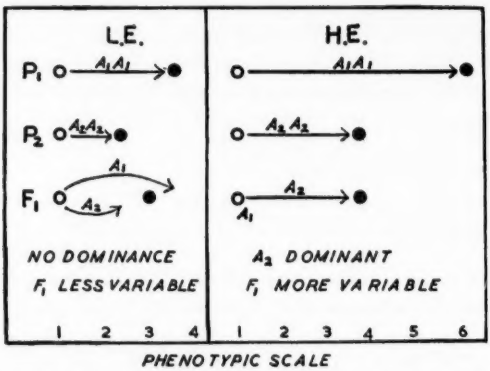

FIG. 8.—Diagram to illustrate the hypothesis of alternative genetic pathways to explain (1) the greater phenotypic stability of heterozygotes to different environments (L.E. and H.E.) and (2) the relationship between high F_1 variability, due to minute uncontrolled developmental effects, and dominance. Note that a less variable F_1 is obtained only when alternative pathways are in operation.

are in parallel which implies similarity but not identity of the genes. This is shown by their similar but not identical phenotypic expressions. If one of the alleles or duplicate genes is dominant or epistatic then it is assumed that the recessive gene pathway is not in operation; if there is no dominance then both pathways are in operation.

This concept of dominance requires that one dose of the dominant allele is able to perform the full function of the two dominant alleles: this has been found to be so for the $\mathbf{R^+}$ gene in *Drosophila* (Stern, MacKnight and Kodani, 1946) where it was possible to compare a hemizygote $\mathbf{R^+}$ with a homozygote $\mathbf{R^+R^+}$. In autotetraploid plants, the general rule is that a gene which is dominant in the diploid is also fully dominant in the simplex form (**Aaaa**) of the tetraploid, thus showing that there is a great margin of safety with dominant genes.

It is also assumed that different genes will in general but not

always have different optima of conditions—temperature, developmental and cytoplasmic environments, etc.—for their operation.

Thus with alternative genetic pathways the effects of changing conditions will be damped ; this will be manifest in greater phenotypic stability to extremes of environment and reduced variability caused by unknown developmental differences. This scheme is illustrated in fig. 8.

In the special case of heterosis there are two main types of gene action to consider based on two different theories of heterosis. There

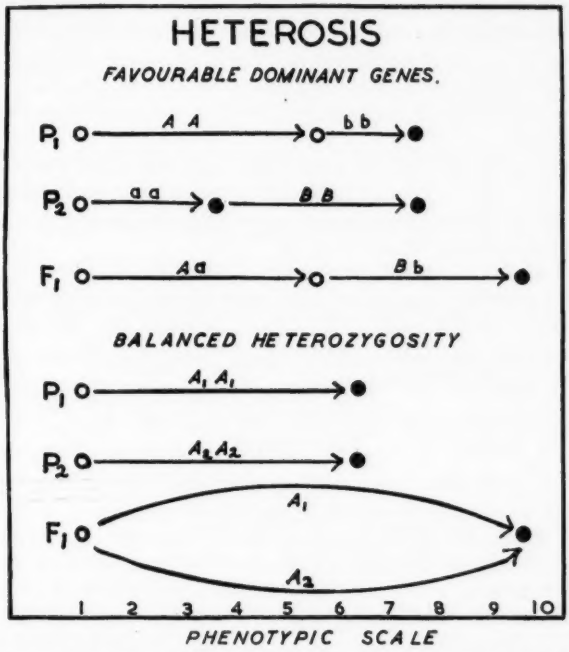

FIG. 9.—Illustrates the two main types of hypothetical gene action which have been postulated for heterosis, only the alternative gene action (balanced heterozygosity) is expected to lead to reduced F_1 variability.

is the theory of *favourable dominant* genes generally attributed to Jones (1917) and there is the *heterozygosity* theory of East (1936) which has been more suitably transformed into the *physiological balance* theory of Rendel (1953).

With the favourable dominant gene theory, the dominant genes concerned in heterosis are working in series. With the physiological balance (heterozygosity) theory they are working in parallel. These two types of action are illustrated in fig. 9.

Thus we would expect F_1 hybrids showing heterosis based on these two methods of gene action to have different relative variabilities.

In the Tomato, *Nicotiana* and *Galeopsis* data, the heterotic hybrids are not less variable than their parents, indicating that heterosis for the characters studied in these plants is due to favourable dominant genes working in series. Evidence of quite a different kind for this type of gene action in heterosis can be found in the results of Smith (*loc. cit.*) in *Nicotiana*, and in *Galeopsis* by Müntzing (1930) where it was shown that heterosis could be fixed in inbred lines. This should be impossible with the physiological balance action without invoking balanced lethals.

Since all the plants quoted which show high variability in heterotic F_1's are inbreeders, it is tempting to think that the favourable dominant type of heterosis may be common in inbreeding species where balanced heterozygosis would be rare. In outbreeding species on the other hand the physiological balance type of heterosis would probably be more frequent.

A model for alternative gene pathways has been demonstrated with major genes in the flagellar antigenic system in *Paramecium* by Beale (1952). Different antigen-controlling genes come into operation under different conditions.

The parallel between the alternative gene pathways and the alternative biochemical synthesis invoked by Hinshelwood to explain biochemical " adaptation " in micro-organisms is obvious. The adaptation can only occur if there are the alternative genetic pathways to carry out the different syntheses.

13. SUMMARY

1. From a simple theoretical model based on a pair of alleles affecting a polygenic character in two environments it is concluded that a heterozygote which is more stable than the homozygotes in respect of the two environments is obtained when one allele is dominant in a particular environment, and when this allele has an effect opposite to that of the environment.

2. Using *Lycopersicon esculentum* as the organism and flower number as the character in two temperature environments, H.E. (High character expression) and L.E. (Low character expression), an experiment with parents, F_1, F_2, F_3, B_1 and B_2 generations has been analysed.

3. Dominance of genes affecting low flower number is almost complete in the H.E. but absent in the L.E. As predicted from the model, this is expressed as greater F_1 phenotypic stability in respect of the two environments.

4. Non-genetic variability of individuals within a single controlled environment due to " intangible environmental effects produced by developmental accidents " is expressed for the F_1 as *relative variability*. This is the standard deviation or the coefficient of variation of the F_1 divided by the mean value of the standard deviation or the coefficient of variation of the parents. A low F_1 relative variability

occurs only when dominance is absent or incomplete. There is a positive linear relationship between F_1 relative variability and the degree of dominance.

5. Published data from *Nicotiana rustica* have been analysed for F_1 relative variability and dominance, and the same relationship is found.

6. F_1 variability is discussed in relation to heterosis, and a theory of *alternative genetic pathways* is advanced which gives some degree of unity to the present data on non-genetic variability, phenotypic stability, dominance and heterosis.

Acknowledgments.—I have pleasure in thanking Mrs P. Dowrick for assistance with the technical work of the experiments and Mr P. Matthews for assistance with the computation and Professor K. Mather, F.R.S. for advice on the lay-out of the main experiment.

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STRUCTURE OF THE INCOMPATIBILITY GENE

IV. TYPES OF MUTATIONS IN *PRUNUS AVIUM* L.

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1. INTRODUCTION

THERE have been two main objects in the work on mutation of the incompatibility gene in *Prunus* :—(1) to investigate the types of spontaneous and X-ray induced mutations, and (2) to produce self-fertile seedlings and from these to breed good self-fertile varieties of fruits.

Previous reports on mutations of the incompatibility gene have been mainly on *Œnothera organensis*. But quantitative data in *Prunus avium* on the amount of seed produced after incompatible pollination with and without X-ray treatment, and calculated mutation rates based on all types of mutation have already been published (Lewis, 1948, 1949). Five years after the pollinations were made the seedlings flowered and have now been tested for their **S** genotypes.

The present account describes the types of mutations obtained spontaneously and after X-ray treatment. The results, some of which have been briefly reported (Lewis and Crowe, 1953), confirm the general conclusions derived from *Œnothera*. In detail, however, the situation is slightly different and has in consequence produced new information about such controversial issues as revertible mutations.

2. MATERIAL AND METHODS

The technique for X-irradiation has been described elsewhere (Lewis, 1949).

The seedling trees to be tested and the horticultural varieties of known **S** genotype used as testers were grown in large pots and brought into an insect-proof greenhouse just before flowering in March. Flowers on trees which were self-fertile or of unknown genotype were emasculated before cross pollination. The degree of compatibility of a pollination was obtained from counts of fruits set at two periods after pollination and counts of seeds produced. The two fruit counts were made because in some pollinations there was a considerable stimulus to fruit swelling which was due to compatible pollen-tube growth, but the majority of zygotes failed to develop owing to zygotic lethals.

The routine method of testing a seedling was to self-pollinate and to cross with its parents in both directions. With critical crosses, the tests were repeated in a second year. The different results and

their interpretations based on these tests are given in table 1. Some of these results, Nos. 2, 3 and 4 are decisive and have only one explanation, but Nos. 1, 5 and 6 each have two alternative explanations which cannot be distinguished without further breeding tests, and these are impracticable in *Prunus* owing to the long period of maturity. Result No. 1 includes homozygotes with the mutated allele having lost its pollen activity and heterozygotes with the mutated allele having lost both pollen and style activities. Result No. 5 is given by homozygotes only. In all of them a pollen mutant has reverted to a normal

TABLE 1

Routine method of testing unknown seedlings for the **S** *alleles (prefix ′ = loss of pollen activity,* o *= loss of stylar activity)*

Pollination	Different results obtained					
	1	2	3	4	5	6
Seedling selfed Parent (S1.2) as ♀ Seedling as ♀	+ + +	+ + −	+ − +	− − −	− − +	− + +
Possible genotype of seedling	S2.2′ S1.1′ S1.2^0 S1^0.2	S1.2′ S1′.2	S1.2^0 S1^0.2	S1.2	S1.1 S2.2 S1^0.1 S2^0.2	S1.*x* S*x*.2 ... ...
Type of mutation	Loss of pollen or both parts	Loss of pollen part	Loss of stylar part	Revertible mutations		New **S** allele or contamination

allele. Some types may in addition have lost their stylar activity but again only further tests in the next generation can distinguish between the mutations affecting 1 or 2 parts of the **S** gene. In result 6 the possibility of a new active allele can only be distinguished from contamination if it is possible to test with all combinations of alleles which are present in the material, including both experimental and neighbouring sources of contamination. With *Prunus* this is an impossible task because only part of the possible sources of contamination are known genetically.

The 6 different classes will be described using the results of a typical case as illustration.

3. RESULTS

Type 1 (*Table* 1)

This type of mutant is self-fertile and compatible both ways with the parent. The behaviour of Seedling 14 11/46 is typical of the group. This plant was obtained from self-pollinating an S3.6 tree without

X-radiation. Self-pollination of the seedling produced 32 fruits from 154 flowers (21 per cent.), when crossed as a male on to the parent 15 fruits were produced from 110 flowers (14 per cent.), and the reciprocal cross gave 27 fruits from 54 flowers (50 per cent.). Thus all the pollinations are fully compatible, since compatible crosses in *Prunus avium* give a set of fruit ranging from 4 to 60 per cent., whereas incompatible crosses give less than 0·3 per cent. (*cf.* Crane and Brown, 1937).

This type of result has two alternative interpretations—(1) that in the mutant allele the pollen activity alone has been changed or (2) that both the stylar and pollen activity have been changed.

Type 2

Seedling 3/45 was obtained from intercrossing two varieties of the incompatibility group III (S3.4), Emperor Francis × Bigarreau Napoleon (X-rayed). Self-pollination of the seedling produced 40 fruits from 200 flowers (20 per cent.). When it was crossed as a male on to the parent 172 fruits were produced from 257 flowers (67 per cent.), while the reciprocal cross gave no fruits from 60 flowers. The failure to set fruit when pollinated with the parent shows that the seedling was a heterozygote and that the stylar activity of the mutated allele is unaltered. The self-fertility and compatibility as a male on to the parent proves that the mutated allele has lost its original pollen activity. This type of mutation is identical with the self-fertile mutants produced in *Œnothera organensis* (Lewis, 1951) and is a confirmation of the bipartite structure of the incompatibility gene in another species.

Type 3

This type of mutant is one of particular interest because it has not been found before. Seedling 6*x* was obtained from Turkey Heart (X-rayed) self-pollinated. Turkey Heart is in the incompatibility group V S3.5. The seedling produced 12 fruits from 295 self-pollinated flowers (4 per cent.) : this is a low set but is within the normal range of compatibility. When crossed as a male on to the parent no fruits were produced from 121 flowers, but in the reciprocal cross 11 fruits were produced from 65 flowers (17 per cent.).

Another seedling of this type 12/45, obtained from Emperor Francis × Bigarreau Napoleon X-rayed, was tested in greater detail. Self-pollinating a total of 402 flowers during four consecutive years has given 25 fruits (6 per cent.), 12 of these dropped from the tree prematurely probably due to embryo abortion. The total result, however, shows that the tree is self-compatible. Crossing as a male on to the parent gave two fruits from 265 flowers (0·8 per cent.), a result which can be classified as incompatible. In the reciprocal cross 20 flowers gave 16 fruits (80 per cent.).

The data suggest that the constitution of the seedlings is either $S_3^0.4$ or $S_{3.4^0}$, in which the 0 suffix denotes a change in the stylar

activity of the S gene. The incompatibility of the seedling pollen with the parental genotype shows that the pollen part of the S gene is unaltered. If this is so the self-fertility can only be due to a mutation in the stylar part of the gene. This possibility is strengthened by the compatibility of the cross between the seedling and parent with the seedling as female. But without further information this could also be due to homozygosity of the seedling. This possibility was excluded by the evidence from the following crosses :

	Flowers	Fruits	Per cent.
Governor Wood ($S_{3.6}$) × 12/45 ($S_{3^0.4}$) .	82	22	27
G. de Hedelfingen ($S_{4.5}$) × 12/45 ($S_{3^0.4}$) .	256	112	44

Thus 12/45 cannot be a homozygote $S_{3.3}$ or $S_{4.4}$ since the pollen is compatible on both $S_{3.6}$ and $S_{4.5}$ styles.

The results of all tests with seedling 12/45 are summarised in table 2.

TABLE 2

Results of test pollinations with seedling 12/45 ($S_{3^0.4}$)

$S_{3.4}$	×	$S_{3^0.4}$	–
$S_{3^0.4}$	×	$S_{3.4}$	+
$S_{3^0.4}$	selfed		+
$S_{3.6}$	×	$S_{3^0.4}$	+
$S_{4.5}$	×	$S_{3^0.4}$	+

The only interpretation which fits these results is that the mutated allele has a changed activity in the style but the original activity in the pollen. The mode of its origin will be discussed later.

Types 4 and 5

These are types which have been found frequently in *Œnothera* and are fairly well understood. They are due to a mutation of the pollen part which has later reverted to normal. An example of type 4 is found in seedling 6 1/46 obtained from Hooker's Black × G. de Hedelfingen (X-rayed). This seedling when selfed produced no

fruits from 228 flowers, when crossed as male on to the parent 166 flowers gave no fruits and the reciprocal cross gave no fruits from 156 flowers. This is clearly a heterozygote like the parent which is the result of a revertible mutation.

Type 5 in which the seedling is self-incompatible, is incompatible as a male but compatible as a female, has two possible interpretations but the most likely is a revertible mutant as in Type 4 but a homozygote.

Type 6

This type is found in seedling 10/45 which was obtained from Late Black Bigarreau S3.5 × Turkey Heart (X-rayed). It produced no

TABLE 3

The numbers of different types of mutations obtained with and without X-rays; owing to a possible loss of identity of one tree it was impossible to classify completely the eight self-sterile seedlings from spontaneous mutation

	Self-fertile		Self-sterile	
	Types 1 and 2	Type 3	Types 4 and 5	Type 6
	Permanent " pollen " loss	Permanent " style " loss revertible " pollen "	Revertible " pollen "	Contamination ?
X-rays . .	3	2	9	10
Spontaneous .	1	1	← 8 →	

fruits from selfing 64 flowers, when crossed on to its parent 480 flowers gave 100 fruits (21 per cent.) and in the reciprocal cross 54 flowers gave 7 fruits (13 per cent.). Thus it is self-incompatible and compatible both ways with the parent. This can only mean either that it was the result of a contamination from a pollen grain from a tree in another incompatibility group or that a new active allele had arisen by mutation.

Seedling 10/45 was tested on a limited scale with some of the possible sources of contamination and it was found to be S3.4 due to a contamination from S3.4 trees which were in the greenhouse at the same time.

With other seedlings of this type it has not been possible to make the tests to exclude contamination as the cause.

FREQUENCIES OF DIFFERENT TYPES OF MUTANTS

Fifty-two seedlings derived from self-pollination, both with and without X-ray treatment, have been analysed either completely or in part. The general policy after making the tests of self-fertility was

to concentrate on the more interesting types : thus many of the seedlings have not been completely classified. Some seedlings showed a low degree of self-fertility or compatibility with the parent, these types are presumably due to mutations similar to those giving complete self-fertility but in which the activity is reduced but not lost. Since the analysis of such types would entail much work and time it was not done.

Table 3 gives the frequencies of the different types which have been completely analysed. From this we can see that the revertible mutations which finally retain the original character of self-sterility are more common than permanent loss mutations which give self-fertility. Furthermore the same range of types, permanent loss and revertible, occur both spontaneously and after X-rays. This is in keeping with the data on *Œnothera organensis* (Lewis, 1951).

The X-ray treatments were given to the pollen-mother-cells at different stages of meiosis. The stage is not synchronised in all the buds receiving treatment, and the number of mutations tested is small which makes a classification of the mutations on the stage of irradiation of doubtful significance. It is suggestive, however, at least as a pointer for future work that the three permanent loss X-ray mutations were from resting stage treatments, while among the revertible mutations, 6 were from resting stage, four from prophase and four from metaphase treatments. If permanent mutations to give self-fertility are required it would appear necessary to include resting stage treatments.

5. DISCUSSION

All but one of the different types of mutations found in *Prunus* are similar to those found in *Œnothera organensis*, and they confirm that the S gene is a complex of at least two parts. The new type of mutation in *Prunus* further confirms this interpretation because it is one which has affected the " stylar part " of the gene. This mutation (type 3) has the pollen activity of the S gene unchanged but has lost its stylar activity. The first sieving test for the mutant allele does not select any " stylar " mutations : it selects only pollen mutants. The chance of obtaining a " stylar " mutation which is independent of a " pollen " mutation is an extremely remote possibility. If, however, a change in the " stylar " part of the gene accompanies a change in the pollen part then the sieve for pollen mutants would at the same time select " stylar " mutants.

In previous publications on *Œnothera*, evidence was presented for the induction by X-rays of revertible mutations of the " pollen " part. Such mutations are of a temporary nature lasting long enough to affect the pollen grain carrying them but reverting before the pollen-mother-cells are formed in the next generation. The type 3 mutants in *Prunus* are important in the interpretation of the revertible mutations because they imply that simultaneous mutations were

induced in both pollen and stylar parts of the gene : the pollen part reverted to normal and the stylar part remained permanently changed. The probable cytological basis for X-ray induced revertible mutation has been pointed out by Lewis and Crowe (1953) and by La Cour and Rutishauser (1953).

Data on the most baffling problem, that of the origin of new active alleles, is so far lacking from *Œnothera*. In *Prunus* it is impossible to investigate this problem owing to the difficulty of identifying contaminations with certainty.

6. SUMMARY

1. X-ray induced and spontaneous mutations of the **S** complex in the sweet cherry, *Prunus avium*, are of three types : (i) permanent loss or change of the pollen activity, (ii) revertible mutation affecting the pollen activity, and (iii) a new type, a permanent loss of the stylar activity.

2. The same range of mutant types are obtained by spontaneous mutation as are induced by X-rays.

3. Permanent loss of either " stylar " or " pollen activity " causes self-fertility. Seedlings which have received only a reverted mutant **S** allele are self-sterile like their parent.

4. All the classes of mutations found in *Œnothera* have also been found in *Prunus*. In addition the new mutation in *Prunus* of the " stylar part " has shown that the two components of the **S** complex (i) can mutate either independently or synchronously and (ii) can revert to normal independently.

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AN ANALYSIS OF QUANTITATIVE VARIABILITY IN *NICOTIANA TABACUM*

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CONFLICTING viewpoints as to the most efficient breeding procedure for improvement of quantitatively inherited characters of normally self-pollinated *Nicotiana* emphasise the need for information concerning the nature of the action of the genes involved. The occurrence of hybrid vigour among first generation hybrids of commercial varieties led to the suggestion of its possible use in practice by Ashton (1946). Smith (1952) favoured the procedure of developing homozygous genotypes through selection since inbreds had been obtained which were superior in many characteristics to the parents or F_1. Knowledge of the additive effects and average level of dominance of the genes controlling the expression of the characters involved would provide a basis for proper evaluation of these and other breeding techniques. The investigation reported here provides estimates of the additive genetic and dominance variance stemming from the actions of genes determining quantitative characters in inter-varietal populations of *Nicotiana tabacum*.

Smith (1952) and Mather (1948) have published estimates of additive genetic and dominance variance for some quantitative characters in *Nicotiana rustica*. Parents, F_1, F_2 and the two first backcrosses provided the genetic material in each of these studies. Both workers report estimates of additive genetic variance of about twice the magnitude of dominance variance for plant height. Smith studied leaf length and node number, reporting ratios of dominance to additive genetic variances of approximately 1 to 5 and 1 to 4, respectively. Apparently these are the only published reports of such information for *Nicotiana*.

Two procedures applicable with plants that are readily cross-pollinated have been used at this station in the estimation of additive genetic and dominance variance. Robinson *et al.* (1949) employed biparental progenies obtained by mating plants of the F_2 generation of a cross of inbred lines and Gardner *et al.* (1953) used similar F_2 material, but obtained progenies by backcrossing each F_2 plant to both of the parent inbred lines used in the original cross. The average level of dominance for genes conditioning yield of corn have all been in the range of over dominance. No more than complete dominance

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has generally been indicated for all other characters. The possible upward bias in the dominance estimate for yield due to repulsion linkages is recognised.

Comstock and Robinson (1948) outlined the procedure used in the investigation reported here, giving the appropriate analysis of variance and genetic composition of the variance components. A comparison of the possible information and relative efficiency of this and the two schemes used with corn has been made by Comstock and Robinson (1952).

It should be pointed out that the general basis for development of the estimation procedures and interpretation of the variance of quantitative characters was provided by Fisher (1918), Fisher *et al.* (1932) and Wright (1935).

I. EXPERIMENTAL PROCEDURE

The genetic design employed in this study is applicable to multi-flowered plants whose inflorescence consists of separate florets as in *Nicotiana*. The F_2 generation of the four inter-varietal crosses of *Nicotiana tabacum* given below formed the foundation stock.

Population 1	Bottom Special × 402
Population 2	Ky 16 × Bottom Special
Population 3	Ky 16 × 402
Population 4	Keliu 49 × 402

Bottom special and 402 are popular high yielding flue-cured varieties adapted to the bright-leaf tobacco producing region of North Carolina. These varieties are different in certain agronomic characteristics and contain some of the most extreme differences in traits observed in the more popular adapted varieties. 402 is a broad-leaf flue-cured type whereas Bottom Special has comparatively narrow leaves. The burley Ky 16 usually is air-cured whereas Keliu 49, grown in Charotar zone, Bombay, India, is sun-cured. The history of the source and development of these varieties is somewhat typical of tobacco varieties in general. Garner (1946) states that the historical facts of varietal origin are usually lacking but that most flue- and air-cured types can be traced to some strains of the Orinoco variety. Bottom Special is considered to be an Eastern N.C. farmer's selection whereas 402 was selected from a cross of 400 × Jamaica. The variety 400 probably originated from a cross of Orinoco and White Burley, according to Garner (1946). Keliu 49 probably traces to American flue-cured types of fairly recent origin. Thus it can be seen that although varieties of very divergent type and area of adaptation were chosen for use in this study, their genetic origin may not be too different.

The F_2 plants of each population were used in groups of eight in 1949 to make $m\,n = 16$ matings consisting of $m = 4$ plants used as male parents and $n = 4$ used as females. This constituted a set of material and ten such sets of 16 matings were attempted in each of the four populations. Insufficient seed for some of the crosses attempted and loss of plants in the seedling stage due to damping-off necessitated use of sets complete for m and n equal to either 3 or 4 in order to utilise as much of the material as possible. The field arrangement was such that each set of progenies compromised a distinct unit of the experiment and the $m\,n$ progenies of the set were planted in a randomised block arrangement with $r = 2$ replications. The total field arrangement of material from a population consisted of s independent units, each unit being a different group of $m\,n$ progenies of m males and n females. Table 1 gives the number of various size sets of progenies and the total number of crosses used in each population.

The seed from each of the 579 crosses was sown on 16th March 1950 in 8-inch pots and sufficient plants for two replications of a 12-plant plot transplanted from each pot to bands in flats as early as possible and subsequently transferred to the field. The field arrangement consisted of contiguous sets of 16, 12 or 9 progenies for each population arranged in randomised blocks with two replications located at the McCullers Experiment Station, 10 miles south of Raleigh, N.C.

Data were collected on the following quantitative characters: Days to flower, plant height, leaf number, leaf length and breadth, total cured leaf yield, and weight of tobacco in each grade as separated by U.S. Government tobacco grader. The days to flower was determined for each plant as the days from seeding to date of bloom of the first flower, at which time the inflorescence was removed from the plant. All height and length measurements were recorded in centimetres and weight in grams. Value per acre, a quantity reflecting both yield and quality, was computed using the 1939-41 three-year average price received for the various U.S. Government grades of all tobacco in the flue-cured region.

TABLE 1

The number of mn *sets and total* F_2 *sib-crosses made in four populations of tobacco*

Size of set ($m \times n$)	Population 1	Population 2	Population 3	Population 4
4×4	8	7	9	6
4×3	1	1	...	3
3×4	...	...	1	...
3×3	1	1	...	1
Total crosses	149	133	156	141

The plot values for all characters except those involving yield and quality were obtained from the data collected on 10 individual competitive plants. Yield and quality determinations were made on plot totals from the same plants and all analyses utilised plot means on a per plant basis. Where less than 10 competitive plants occurred, plot values were based on the number of such plants available. Individual plant data from approximately every tenth plot provided estimates of "plants in plots" mean squares.

Variances in the data were analysed as shown in table 2. Genetic interpretation of the variance components arising from progeny differences in this type of an experiment have been given by Comstock and Robinson (1948) and (1952). Assumptions involved in deriving these genetic interpretations are discussed in detail with the conclusion that the assumptions most likely of questionable validity are (1) no linkage among segregating genes affecting the character in question, and (2) no epistasis, *i.e.* the effect of variation in the genotype at any single locus is not modified by genes at other loci. Consequences of their invalidity and some discussion of the magnitude of bias resulting from these assumptions can be obtained from Comstock and Robinson (1952). With these assumptions it can be shown that

$$\sigma_m^2 = \sigma_f^2 = \Sigma q_i(1-q_i)[1+(1-2q_i)a_i]\frac{u_i^2}{2} = 1/4\ \sigma_g^2$$

$$\sigma_{mf}^2 = \Sigma q_i(1-q_i)a_i^2u_i^2 = 1/4\ \sigma_d^2$$

where u_i is one-half the difference in effect between the two homozygous genotypes of the i^{th} segregating locus, a_iu_i the deviation of the effect of the heterozygous genotype from the mean effect of the homozygous genotypes, q_i the frequency of the favourable allele, and σ_g^2 and σ_d^2 denote additive genetic and dominance variance,

respectively. The magnitude of a_i measures the degree of dominance in the action of the i^{th} pair of genes as follows :

Magnitude of a_i	*Degree of dominance*
$a_i = 0$	No dominance
$0 < a_i < 1 \cdot 0$	Partial dominance
$a_i = 1 \cdot 0$	Complete dominance
$a_i > 1 \cdot 0$	Overdominance

A weighted mean of the a_i^2's, weighting being relative to the u_i^2's, is

$$\bar{a}^2 = \frac{\sum_i a_i^2 u_i^2}{\sum_i u_i^2} = \frac{2\sigma_{mf}^2}{1/2(\sigma_f^2 + \sigma_m^2)}$$

and

$$\bar{a} = \sqrt{\frac{2\sigma_{mf}^2}{1/2(\sigma_f^2 + \sigma_m^2)}}$$

which provides a measure of the average dominance of the genes concerned if the gene frequency of the population is 1/2 which was the assumption for the material used here.

TABLE 2

General form of the analysis of variance obtained from each population of F_2 sib-crosses of tobacco

Source of variation	d.f.	M.S.	Expectation of M.S.
Sets	$s-1$	...	...
Replications in sets . .	$s(r-1)$	...	...
Males in sets . . .	$\sum_i (m_i - 1)$	M_1	$\sigma^2_{w/k'} + \sigma^2 + r\sigma^2_{mf} + rn'\sigma^2_m$
Females in sets . . .	$\sum_i (n_i - 1)$	M_2	$\sigma^2_{w/k'} + \sigma^2 + r\sigma^2_{mf} + rm'\sigma^2_f$
Males × females in sets .	$\sum_i (m_i - 1)(n_i - 1)$	M_3	$\sigma^2_{w/k'} + \sigma^2 + r\sigma^2_{mf}$
Remainder among plots .	$\sum_i (m_i n_i - 1)(r-1)$	M^4	$\sigma^2_{w/k'} + \sigma^2$
Plants in plots . . .	$\sum_i \sum_j (k_{ij} - 1)$	M^5	σ^2_w

s = the number of sets of progenies

r = the number of replications

m_i = the number of male parents in the i^{th} set

n_i = the number of female parents in the i^{th} set

k_{ij} = the number of plants in i^{th} plot of the i^{th} set

$$m' = \frac{\sum_i m_i(n_i - 1)}{\sum(n_i - 1)} \qquad n' = \frac{\sum_i n_i(m_i - 1)}{\sum(m_i - 1)}$$

σ^2_w = the intra-plot variance

σ^2 = the inter-plot error variance arising from intra-replication plot effects

σ^2_{mf} = the progeny variance due to interaction of the genotype of male and female parents

σ^2_f = the progeny variance due to genetic differences among female parents

σ^2_m = the progeny variance due to genetic differences among male parents

Estimates of the variance components are obtained from the analysis in table 2 as follows :

$$\hat{\sigma}^2_{mf} = 4(M_3 - M_4) = \hat{\sigma}^2_d.$$

If the material in the experiment had been complete as designed, then $m = n$ and M_1 and M_2 could have been pooled since each of the mean squares have the same expectation. Since $m \neq n$ in this study, $\hat{\sigma}^2_m$ and $\hat{\sigma}^2_f$ are computed separately as

$$\hat{\sigma}^2_m = \frac{M_1 - M_3}{rn} \text{ and } \hat{\sigma}^2_f = \frac{M_2 - M_3}{rm}$$

and

$$\hat{\sigma}^2_g = 2(\hat{\sigma}^2_m + \hat{\sigma}^2_f).$$

Covariance analyses between all pairs of characters, similar to that illustrated for the variance analysis in table 2, were computed and from these the completely analogous covariance components were estimated.

TABLE 3

Estimates of the additive genetic variance, $\hat{\sigma}^2_g$, and their standard errors calculated for various characters in tobacco *

Character studied	Population 1	Populations 2 and 3	Population 4	Populations combined
Days to flower .	2·50±0·67	4·49±1·15	3·46±1·05	3·72±0·61
Plant height .	39·83±11·48	46·54±13·60	53·66±15·68	46·40±8·02
Leaf number .	0·5782±0·2191	0·8814±0·1800	1·0846±0·3150	0·8526±0·1296
Leaf length .	1·8694±0·6630	0·8966±0·5589	2·1650±0·9849	1·46±0·3934
Leaf breadth .	3·7198±1·1494	2·0590±0·5119	2·9660±0·9516	2·70±0·4354
Leaf yield .	44·50±14·84	49·19±15·34	30·98±15·11	43·58±9·14
Leaf value .	661·95±241·22	890·10±266·81	562·68±264·49	744·97±157·34

* S.E. $\hat{\sigma}^2_g = \sqrt{4\left\{\frac{\frac{2(M_1)^2}{\sum_i (m_i-1)} + \frac{2(M_3)^2}{\sum_i (m_i-1)(n_i-1)}}{(rn')^2} + \frac{\frac{2(M_2)^2}{\sum_i (n_i-1)} + \frac{2(M_3)^2}{\sum_i (m_i-1)(n_i-1)}}{(rm')^2}\right\}}$

2. RESULTS

Tables 3 and 4 present the estimates of additive genetic variance, $\hat{\sigma}^2_g$, and dominance variance, $\hat{\sigma}^2_d$, respectively. Since populations 2 and 3 both involve the two flue-cured parents crossed to the same burley variety, Ky 16, combined estimates from these populations are presented.

The marked morphological differences between parental varieties involved in the flue-cured × burley and flue-cured × Indian Sun-cured populations suggested the possibility of more genetic variability among the segregating progenies from these populations than among progenies derived from crossing two flue-cured varieties widely grown in the same region of adaptation. The results are not in accord with this expectation. The estimates of additive genetic variance are quite similar for all characters for the three groupings of genetic material. The standard errors associated with each of the estimates provide a basis for evaluating the reliability of these estimates, but

are not exact when used for setting fiducial limits. The fiducial limits about these estimates are not symmetrical but will generally be higher than the lower or upper limits indicated by use of these standard errors. Approximately double the number of progenies is involved in the combined estimates calculated for populations 2 and 3, resulting in smaller standard errors than those obtained for individual populations. Combining all material reduces the standard errors to approximately one-third to one-fourth the estimated σ_g^2.

The magnitude of the dominance variance estimates is strikingly different from the additive genetic variances. Nineteen of the twenty-eight " males × females in sets " mean squares (M_3, table 2) were smaller than the error mean square although the expectation of M_3

TABLE 4

Estimates of the dominance variance, $\hat{\sigma}_d^2$, and their standard errors calculated for various characters in tobacco *

Character studied	Population 1	Populations 2 and 3	Population 4	Populations combined
Days to flower .	0·53±0·76	0·34±1·91	(−2·13±1·64)	(−0·22±0·96)
Plant height .	(−17·30±14·86)	8·61±24·87	(−8·53±21·99)	(−2·46±13·29)
Leaf number .	(−0·1170±0·4308)	0·0468±0·2400	(−0·3760±0·4560)	(−0·0964±0·1960)
Leaf length .	0·5160±1·1552	0·4892±1·5616	(−0·0110±2·1008)	0·36±0·9484
Leaf breadth .	(−1·0678±1·7280)	0·3048±0·8680	0·4844±1·3780	(−0·02±0·6984)
Leaf yield .	(−55·84±29·53)	(−16·06±34·17)	(−33·11±36·34)	(−30·68±20·19)
Leaf value .	(−899·41±510·68)	(−240·35±569·92)	(−426·95±652·00)	(−459·97±344·52)

* S.E. $\sigma_d^2 = \sqrt{\dfrac{\dfrac{2(M_3)^2}{\sum_i (m_i-1)(n_i-1)} + \dfrac{2(M_4)^2}{\sum_i (m_i n_i-1)(r-1)}}{r^2}}$

contains the additional dominance variance component. An indication of the variability of estimates of σ_d^2, computed from both negative and positive components of variance, is provided by the values in table 4. In no case did the negative values deviate sufficiently from zero by more than was accounted for by sampling variation as shown by the standard errors. The only biological explanation offered for the high number of negative variance components is the possible existence of negatively correlated effects between adjacent plots. In the cases of yield and value determinations, no positive components of variance were found for any of the populations. All other characters in the flue-cured-burley populations did provide some indication of dominance variance but here the standard errors are larger than the estimates in every case. The estimate of σ_d^2 from all populations combined is positive only for leaf length ; however, the associated standard error indicates this may be no different from zero. The null hypothesis of no dominance variance was tested from the analyses of variance in terms of $F = M_3/M_4$. In no case was the F significant

at the 0·05 probability level. Thus the data provide no statistically significant evidence for dominance at any level.

The presence of additive genetic variance indicates that selection among these populations should be effective. Let G_s be the expected difference in mean genotypic merit between a population obtained by random interbreeding among selected progenies and the population in which selection was practised. This difference may be taken as a measure of the expected effect of selection and can be expressed as

$$G_s = K\sigma_c \frac{\sigma_y^2}{\sigma_c^2} = \frac{K\sigma_y^2}{\sigma_c} \quad . \quad . \quad . \quad (1)$$

where, for the character under selection,

σ_y^2 = the additive genetic variance among selection units ; $1/2\sigma_g^2$ in the case of these full-sib progenies,

σ_c^2 = phenotypic variance among progeny values on which selection is based (for selection among these progenies based on the means for plots in two replications

$$\sigma_c^2 = \frac{\sigma_{w/k}^2 + \sigma^2}{r} + \sigma_{mf}^2 + \sigma_m^2 + \sigma_f^2$$

K = the selection differential in multiples of σ_c.

It is frequently desirable to compute G_s as a percentage of the population mean. For this purpose we can write

$$\frac{(100)Gs}{\bar{x}} = \frac{(100)K\sigma_y^2}{\bar{x}\sigma_c} = K\left(\frac{100\sigma_y}{\bar{x}}\right)\left(\frac{\sigma_y}{\sigma_c}\right) \quad . \quad . \quad (2)$$

Note that if the only variance among progeny values were that represented in σ_y^2, *i.e.* if $\sigma_c^2 = \sigma_y^2$, expected progress, under constant selection intensity as represented by K, would depend only on $(100\,\sigma_y/\bar{x})$. Burton (1952) defined the latter as the genetic coefficient of variability and (recognising that $\sigma_y/\sigma_c \leqslant 1{\cdot}0$) pointed out that it reflects the limit in progress from selection in a single generation that can be approached through minimising the non-genetic portion of σ_c^2. Genetic coefficients of variability for full-sib progenies are presented in table 5. They were computed as

$$100\sqrt{\frac{\hat{\sigma}_g^2/2}{\bar{x}}}$$

using estimates of σ_g^2 from table 3. Also presented in table 5 are estimates (computed in terms of equation 2) of progress from selection among the full-sib progenies in terms of means for the two replications used in the study. K was set equal to 2·06, its expected value assuming 5 per cent. selection from a large sample out of a normally distributed population of progenies. In view of population-to-population consistency in estimates of $\hat{\sigma}_g^2$ (table 3) only values obtained using the combined data of all four populations are presented.

Values for estimated progress range from about equal to about 40 per cent. greater than the corresponding coefficient of genetic

variability. Since they would have been 2·06 times as large assuming equality of σ_y^2 and σ_e^2, this reflects the fact that values of $\sqrt{\sigma_y/\sigma_e}$ were in the range 0·49 to 0·68.

3. DISCUSSION

Any interpretation of these results must be conditioned by the possible bias due to genotypes giving differential response due to environmental changes. Since this study was conducted in only one location and year, variance due to interaction of genotypes with the environment of the particular location and year involved would contribute to the apparent genetic variance and the estimates of the latter are correspondingly biased as measures of variance in genotypic merit with respect to the whole population of environments for which improvement in the crop is desired. It follows that the estimates of

TABLE 5

Genetic coefficients of variability and estimates of progress for various characters with all populations combined

Character studied	Genetic C.V.	Estimates of progresss
Days to flower . .	1·03	1·40
Plant height . . .	3·25	4·31
Leaf number . . .	2·93	4·12
Leaf length . . .	1·55	1·56
Leaf breath . . .	3·59	4·93
Leaf yield . . .	4·60	5·09
Leaf value . . .	5·44	6·04

progress through selection may also be biased upward. The data provide no information on the possible magnitude of variance due to interaction of genotype with location and year. Results obtained at this institution with other crops (largely unpublished) suggest that at most it probably does not exceed that of the actual genetic variance.

While an expected advance of about 5 per cent. from selection among these progenies for leaf yield is suggested, there can be no guarantee that comparable gains could also be made in following generations. It is to be expected that improvement rate will decline as selection is continued. On the other hand unless the number of genes involved is considerably smaller than ordinarily assumed for such quantitative characters it would appear entirely possible that as much as 15 or 20 per cent. improvement might be effected by continuing selection over several generations, selected progenies in each generation being interbred (rather than selfed) to yield the next generation. Such a conclusion is strongly supported by the coefficient of genetic variation when computed on the basis of single plants rather than progenies. For example, for leaf yield it is found to be 6·5 per cent. This suggests that there were plants in these

populations that were as much as 15 per cent. superior in genotype (2·5 standard deviations above the mean) to the population average and hence that an entire population equally as good is not impossible. The results for leaf size characters indicate that much more rapid progress is possible in these populations when selecting for strains with broader leaves than for strains having longer leaves.

The genetic correlations among characters become of extreme practical importance when the economic evaluation of a crop depends upon two or more traits. This is especially true of tobacco where improvement in yield must be accompanied by acceptable quality. The genetic correlation among all pairs of characters were determined but only those computed from the population developed from crossing flue-cured varieties and pertinent to this discussion will be mentioned.

These genetic relationships indicate that selecting higher yielding progenies from this population will, on the average, result in concurrent improvement in all yield components studied—plant height,

TABLE 6

Genetic correlations between leaf yield and other characters in population 1

Days to flower	Plant height	Leaf number	Leaf length	Leaf breadth	Leaf value
0·1280	0·2980	0·6083	0·4419	0·4897	1·0042

leaf number and leaf size. The expected progress in leaf yield when the superior 5 per cent. of the progenies are selected on the basis of high leaf number was computed to be 62 per cent. as much yield improvement as when selection was for yield itself. The basis of computation has been given by Robinson and Comstock (1952). This is a practical illustration of the possible usefulness of information on the genetic relationships between characters. Selection would be indirectly for higher yield but based on leaf count, a more readily determined value. The magnitude of this relationship was not consistent for all populations, being quite low for population 4. This indicates that maximum progress from selecting for two or more characters simultaneously must be based upon a knowledge of the genetic inter-relationships of the characters for the specific material involved. A very high genetic correlation between yield and value is noted in table 6 and this same relationship existed in the other populations. It is not suggested that this would be universally true in such populations from variety crosses within *Nicotiana tabacum*. Should tobacco varieties with sufficiently different attributes of quality be involved as parents of such populations, this genetic correlation may be considerably reduced. Furthermore, it is not implied from these results that better quality tobacco is associated with the higher yielding progenies. Actually the price per hundred pounds of tobacco

decreased as yield increased in all of the populations. Nevertheless, these results indicate that selection for yield among progenies of segregating populations developed from parental varieties with acceptable quality characteristics need not be subjected in the early stage of strain development to the expensive procedure of curing and grading for the purpose of arriving at a "value" rating. The additional effort and cost can probably best be used in evaluating more progenies for yield under different environmental conditions.

The low estimates of any dominance effects in the action of the genes conditioning the expression of the various characters is of particular importance in the consideration of breeding procedures. Since epistatic effects would contribute to dominance variance estimates it is further assumed that they too are relatively unimportant in the action of genes considered in these populations. Furthermore, recognising that high frequency of repulsion phase linkage is a possible source of upward bias in the dominance variance, assuming dominance is associated with the favourable allele only, gives additional support to the evidence of negligible dominance. Smith (1953) came to the same general conclusions from results obtained in *Nicotiana rustica*, even though more dominance was reported than was obtained in this study. However, when the sampling errors attached to his estimates are considered, in no case was dominance variance significantly different from zero. The observation of appreciable heterosis in F_1 hybrids of *Nicotiana tabacum* variety crosses is contrary to expectation based on the degree of dominance indicated in this study. Patel (1949) compared the F_1 with mid-parent values for the same populations used in this study and reported heterosis to the extent of 2 and 6 per cent. for leaf yield and plant height respectively. Mann (unpublished) tested six flue-cured varieties and the 15 possible F_1 hybrids at three locations in 1952 and found a 2·5 per cent. difference in yield in favour of hybrids which was non-significant. The low amount of heterosis suggests at least some degree of dominance but probably low in view of these results and in accord with Smith's conclusion.

4. SUMMARY

This investigation provides estimates of the additive genetic and dominance variance in four F_2 populations developed from inter-varietal crosses in *Nicotiana tabacum*. The scheme for producing the experimental progenies is appropriate with plants with multiple inflorescence which requires a set of *mn* crosses among sets of *m* individuals used as male parents with *n* female parents.

The results indicate that little dominance is concerned in the action of genes conditioning the inheritance of the characters considered. The low magnitude of the estimates of dominance variance indicates little effect from epistasis which would tend to produce upward bias. The estimates of additive genetic variance were of

sufficient magnitude to suggest appreciable progress in the improvement of most of the characters studied from selecting among the progenies.

Genetic coefficients of variability were computed and inferences made from these as to the limit of progress from selection. Indications are that approximately 15 per cent. advance in leaf yield could be effected by creating a new population from the most superior genotype.

Genetic correlations between leaf yield and several other characters in population 1 are presented and implications given of the consequences of such relationships on practical breeding problems. The extremely high positive genetic correlation between leaf yield and value of cured leaf is the basis for suggesting that determination of the latter trait could be omitted in breeding work with certain material.

The limitations of these results are emphasised with respect to possible bias from genotype-environmental interactions. If these interactions are important the estimates obtained in this study under one environment may not provide a realistic appraisal of rate of improvement possible. However, assuming them to be applicable for other environments and for similar type populations of *Nicotiana tabacum* it is concluded that a breeding procedure designed to accumulate the maximum number of favourable genes in homozygous genotypes should offer more promise for tobacco improvement than attempting to utilise F_1 hybrids on a commercial scale. The possibility of about 10 per cent. improvement in yield above the population mean is suggested from developing true breeding strains from selfing and selecting in this material. Additional progress can only result from recombination of selected genotypes.

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GENE FREQUENCIES IN WILD POPULATIONS OF *TRIFOLIUM REPENS*

II. DISTRIBUTION BY ALTITUDE

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1. INTRODUCTION

A PREVIOUS investigation (Daday, 1954) was concerned with the distribution of the glucoside lotaustralin and enzyme linamarase gene frequencies in *Trifolium repens* populations derived from locations at low altitudes in Europe and the Near East. As the location of origin of populations moved from the Mediterranean region to north-eastern Europe, a progressive diminution in the frequency of the dominant alleles was found. This diminution ranged from 100 per cent. to nil. A correlation was also found to exist between decreasing gene frequencies and decreasing January mean temperatures. It is known that the presence and absence of glucoside and enzyme is determined by two separate pairs of genes—*Ac-ac* and *Li-li* respectively (Corkill, 1942). The phenotypic frequency was calculated from the proportions of plants having the four chemical phenotypes : (1) Glucoside and enzyme (*AcLi*) ; (2) Glucoside only (*Acli*) ; (3) Enzyme only (*acLi*) ; (4) Neither glucoside nor enzyme (*acli*). The genotypic structure was determined by means of the Hardy-Weinberg formula ($p^2+2pq+q^2 = 1$). The aim of the present investigation was to study the genetical structure of *T. repens* populations derived from different altitudes in the Central European Alps. For this purpose a collection of wild *T. repens* seed was obtained from Switzerland, together with a sample from Austria.

2. METHOD

The modified Guignard picric acid test was used to distinguish between the four phenotypes of *T. repens*. This method described by Corkill (1940) was applied, together with an additional test. The seed was sown in boxes in a greenhouse, and the picric acid test was carried out when the plants were two to three months old, using solutions of linamarase (prepared according to the method of Coop, 1940) and a standardised solution of isolated lotaustralin (Melville and Doak, 1940). The presence and intensity of the colour reaction was recorded and graded into six groups.

The method described by Corkill (1940) was extended by making an additional test on the *Acli* phenotype. Plants of the *Acli* phenotype were further tested with lotaustralin. Two drops of solution with chloroform were added to freshly collected leaf samples in a test tube.

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After 18-20 hours' incubation many plants showed a colour reaction on the picric acid paper. Plants of the *Acli* phenotype which reacted positively were classified as *AcLi* phenotype, while plants which gave no reaction to this test remained in the *Acli* phenotype group. These reactions in most cases were very weak, but they indicated that many plants designated *Acli* phenotype by the first test may also contain a small amount of enzyme, the quantity being so small as to be insufficient to hydrolise the cyanogenetic glucoside in the plants.

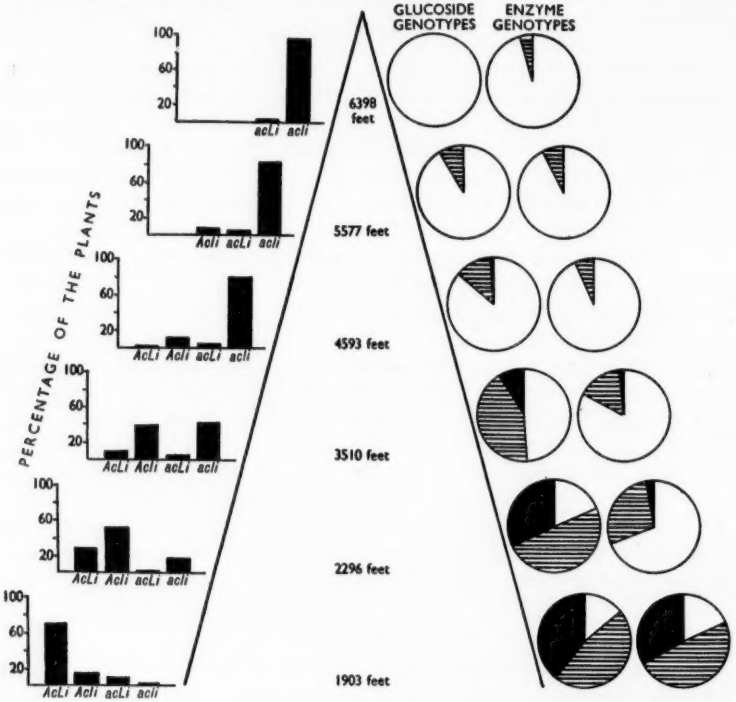

FIG. 1.—Phenotypic and genotypic frequencies in wild populations of *Trifolium repens* from different altitudes.

Phenotypes (left) :
AcLi = glucoside and enzyme
Acli = glucoside only
acLi = enzyme only
acli = neither glucoside nor enzyme

Genotypes (right) :
Black section = dominant homozygotes
Lined section = heterozygotes
White section = recessive homozygotes

Variation in enzyme gene action suggests the presence of a gene with very low enzyme output, which could not be identified without applying this additional test. The picric acid test was found to be suitable for estimating the enzyme output by using a standard solution of isolated lotaustralin. In some samples the proportion of this type of plant exceeded 20 per cent.

3. GENETICAL STRUCTURE OF THE WILD POPULATIONS

The first indication of differences between the gene frequencies of populations from high altitudes and those from low altitudes was observed after testing a small sample of *T. repens* collected by Beddows (1949) from the Pyrenees (Gavarnie—altitude about 5000 feet). Tests of this sample showed the presence of a preponderance of the *acli* phenotype as compared with French samples from low altitudes, which consisted mainly of plants of the *AcLi* phenotype.

In the present investigations, *T. repens* samples were collected from regions of different altitudes in the Central European Alps. The results of analyses of the phenotypic and genotypic structure of these samples are presented in tables 1 and 2 and in fig. 1. Lausanne, situated beside Lake Geneva at 1903 feet a.s.l., was the lowest location from which samples were collected.

TABLE 1

Frequency (per cent.) of the four phenotypes in wild populations of Trifolium repens *from different altitudes*

Localities	Altitude in feet	*AcLi*	*Acli*	*acLi*	*acli*	No. of plants
Lausanne . .	1903	70·65	15·22	10·87	3·26	184
Naters . . .	2296	28·00	53·00	2·00	17·00	100
Fiesch . . .	3510	10·91	40·00	6·36	42·73	110
Alpage de Crausey .	4593	1·11	12·22	4·45	82·22	90
Kreuz . . .	5577	0·00	8·51	7·45	84·04	94
Gross Glockner .	6398	0·00	0·00	4·04	95·96	99

Collecting points at higher altitudes were as follows :—Naters (2296 feet), Fiesch (3510 feet), Alpage de Crausey in Canton Valais (4593 feet), Kreuz in Canton Grison (5577 feet) and Gross Glockner in Salzburg (6398 feet). The Lausanne sample was typical of the Swiss lowlands, and no altitudinal effects were observed. Over two-thirds of this population consisted of the *AcLi* phenotype ; the *Acli* and *acLi* phenotypes were present in the small proportion of about 15·22 and 10·87 per cent., while the *acli* phenotype constituted only 3·26 per cent. of this population. If the proportions of the four phenotypes in the populations from the six different altitudes are compared, marked differences are apparent. In the sample from Naters (2296 feet) the percentage of the *AcLi* phenotype drops to less than one half of that in the population from Lausanne (1903 feet). At 3510 feet (Fiesch) and 4593 feet (Alpage de Crausey) the proportions of the *AcLi* phenotype in the populations are 10·91 and 1·11 per cent. respectively. The populations at the two highest altitudes contain no *AcLi* phenotype.

The distribution of the *Acli* phenotype in Alpine populations may be summarised as follows. In contrast to the low frequency of *Acli* at 1903 feet, 53·00 per cent. of the sample from 2296 feet was of this

phenotype. As the altitude increases—Fiesch (3510 feet), Alpage de Crausey (4593 feet), Kreuz (5577 feet)—the percentage of *Acli* again declines ; at 6398 feet this phenotype is completely absent. There is little variation in the proportion of the *acLi* phenotype, which is present in a low percentage in all the Alpine populations studied.

TABLE 2

Frequency of the glucoside and enzyme genotypes in wild populations of Trifolium repens *from different altitudes*

Localities	Glucoside genotypes			Enzyme genotypes		
	p^2	$2pq$	q^2	p^2	$2pq$	q^2
	AcAc	*Acac*	*acac*	*LiLi*	*Lili*	*lili*
Lausanne . .	0·390	0·469	0·141	0·325	0·490	0·185
Naters . . .	0·318	0·492	0·190	0·027	0·273	0·700
Fiesch . . .	0·089	0·420	0·491	0·008	0·165	0·827
Alpage de Crausey .	0·005	0·128	0·867	0·001	0·055	0·944
Kreuz . . .	0·002	0·083	0·915	0·001	0·073	0·926
Gross Glockner .	0·000	0·000	1·000	0·0004	0·0400	0·9596

As altitude increases, there is a corresponding increase in the percentage of the *acli* phenotype from 3·26 at Lausanne to 95·96 per cent. at Gross Glockner. As previously reported in the lowland populations of Europe there is here further evidence of a change in frequency of the different phenotypes with changing environmental conditions.

TABLE 3

Frequency of the glucoside lotaustralin and enzyme linamarase genes in populations of Trifolium repens *from different altitudes*

Localities	Altitude in feet	Glucoside gene		Enzyme gene		January mean temperature °F.
		Ac	*ac*	*Li*	*li*	
Lausanne . .	1903	0·6241	0·3759	0·5701	0·4299	30·10
Naters . . .	2296	0·5642	0·4358	0·1633	0·8367	29·30
Fiesch . . .	3510	0·2994	0·7006	0·0904	0·9096	25·70
Alpage de Crausey .	4593	0·0690	0·9310	0·0282	0·9718	28·40
Kreuz . . .	5577	0·0435	0·9565	0·0380	0·9620	24·80
Gross Glockner .	6398	0·0000	1·0000	0·0204	0·9796	20·30

The genotypic structure of the populations is presented in table 2. The frequencies of the genotypes *AcAc*, *Acac*, *acac*, and *LiLi*, *Lili*, *lili* were calculated by means of the Hardy-Weinberg formula. About one-third of the population from Lausanne has the *AcAc*, *LiLi*, genotypes, and nearly half of the sample appears to be heterozygous (*Acac*, *Lili*), while *acac*, *lili* occur at a frequency of 0·141 and 0·185

respectively. The frequency of both the dominant homozygous genotypes decreases progressively throughout the whole series as altitude increases. Simultaneously the proportion of the *acac* and *lili* genotypes increases until plants of populations existing at the Gross Glockner location (6398 feet) are entirely *acac*, and the *lili* genotype shows a frequency of 0·9596. These findings provide evidence of the changes occurring in phenotypes and genotypes of wild populations of *T. repens* at different altitudes.

Table 3 and fig. 2 represent the allele frequencies in wild *T. repens* populations of the Central European Alps. A gradual decrease was found in the proportion of dominant glucoside lotaustralin (*Ac*) and enzyme linamarase (*Li*) alleles. Consequently both recessive alleles

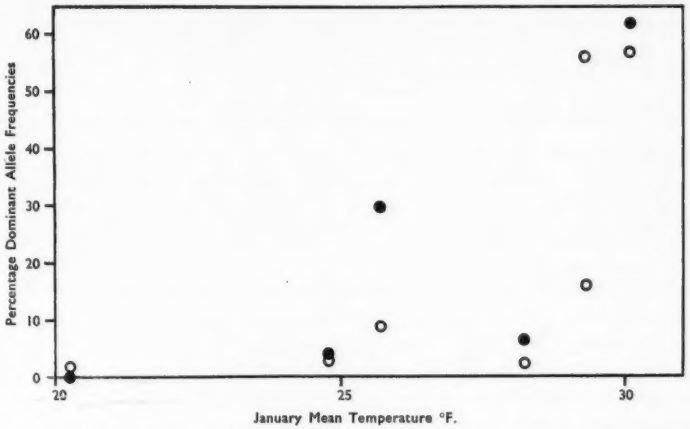

FIG. 2.—Relation between the January mean temperature and both the frequencies of the dominant glucoside lotaustralin (*Ac*) and enzyme linamarase (*Li*) alleles in populations of *Trifolium repens* from different altitudes.
● = dominant glucoside allele. ○ = dominant enzyme allele.

(*ac*, *li*) increased as altitude increased and as the January mean temperature decreased.

Melville *et al.* (1940) established that the picric acid test is not only qualitative but also provides a rough quantitative measure. The six grades of colour reaction were found to correspond to an increase from about 0·006 to 0·0335 per cent. HCN content in the fresh leaf material. The frequency of the six reaction groups are given in table 4.

From this table it is apparent that the largest proportion of plants from Lausanne, Naters and Fiesch populations fall into reaction groups 4, 5 and 6, the greatest proportion falling into group 5, though the enzyme reaction of the Fiesch population is an exception. A large majority of plants from the other populations fall into the first three reaction groups. High frequency of occurrence of the dominant

alleles appears to be correlated with a high output of glucoside enzyme products at low altitudes. In contrast, *T. repens* populations from higher slopes and which exhibit lower dominant gene frequencies have, in general, a lower output of gene products.

4. DISCUSSION

Variability within the species in response to diverse environmental conditions at different altitudes was established in *Potentilla glandulosa* (Clausen *et al.*, 1940). On the basis of morphological and ecological

TABLE 4

Frequency of the picric acid paper reactions in populations (in per cent. of the plants bearing glucoside or enzyme) from different altitudes

Localities	Altitude in feet	No. of plants bearing glucoside	Glucoside reaction					
			1	2	3	4	5	6
Lausanne . .	1903	158	1·9	8·2	12·7	8·9	*56·3*	12·0
Naters . . .	2296	81	5·0	7·4	11·1	11·1	*33·3*	32·1
Fiesch . . .	3510	56	1·8	0·0	10·7	17·8	*50·0*	19·7
Alpage de Crausey .	4593	12	0·0	25·0	*58·3*	16·7	0·0	0·0
Kreuz . . .	5577	8	25·0	0·0	*62·5*	0·0	12·5	0·0
Gross Glockner .	6398	0	0·0	0·0	0·0	0·0	0·0	0·0

Localities	No. of plants bearing enzyme	Enzyme reaction						No. of tested plants
		1	2	3	4	5	6	
Lausanne . .	150	4·6	6·0	12·7	5·3	*46·7*	24·7	184
Naters . . .	30	13·4	26·6	3·3	10·0	*30·0*	16·7	100
Fiesch . . .	19	*37·0*	21·1	15·7	15·7	10·5	0·0	110
Alpage de Crausey .	5	20·0	*60·0*	20·0	0·0	0·0	0·0	90
Kreuz . . .	7	14·3	28·6	*42·8*	0·0	14·3	0·0	94
Gross Glockner .	4	*75·0*	0·0	0·0	0·0	25·0	0·0	99

characters, four subspecies were distinguished in that species originating from the central Californian transect. Within each subspecies further differentiation into climatic races was observed. Other work of Clausen *et al.* (1948) described the altitudinal climatic races of *Achillea* from a west to east transect across central California. Dobzhansky (1948) found that differences in the gene arrangement of the third chromosome occurred in populations of *Drosophila pseudoobscura* and *Drosophila persimilis* from Sierra Nevada, California. Out of the seven different gene arrangements, the Standard is the most frequent at low altitudes, but becomes progressively less frequent at higher altitudes of the mountain range. The Arrowhead gene arrangement is most frequent at the subalpine zone and decreases in frequency down the slope. Six gene arrangements were found in the third chromosomes

of *Drosophila persimilis*. The Whitney gene arrangement was more common in populations at low than at high altitudes; other gene arrangements show a reverse trend. The results obtained from *T. repens* also show a change in genetical composition with elevation. Both dominant glucoside lotaustralin and enzyme linamarase allele frequencies decrease with increasing altitude from 1903 to 6398 feet in the Central European Alps. This gene frequency cline shows a corresponding gradation with the January mean temperature (table 3). Detailed analyses of European populations indicated a significant relationship between changes in gene frequency and decreasing January mean temperature (Daday, 1954). A correlation between gene frequency and altitude is therefore not unexpected.

Some explanation is necessary in respect of the relation between January mean temperature and gene frequency at Alpage de Crausey. This place is situated at a considerably higher altitude (4593 feet) than Fiesch (3510 feet), but the January mean temperature is higher, being 28·40° F., compared with 25·70° F. at 3510 feet. In general, temperature declines with increasing altitude, and this reversal of temperature conditions at Alpage de Crausey must be attributed to local geographical conditions. In spite of this, however, the dominant allele frequency remains lower at 4593 feet than at 3510 feet; this may be due to the microclimate of Alpage de Crausey where the sample was collected.

A divergence in the action of genes has also been shown by Raper (1927) in investigations on colour differences in mammalian hair. The pigmentation of hair is due to the presence of melanin, which is derived from tyrosine by means of its oxidation into dihydroxyphenylalanine, this being then converted into pigment in the presence of an enzyme. Other studies (Russell, 1939) indicate that gene replacement at other loci influences the quantity of pigment by affecting the activity of the enzyme system, thus producing various degrees of hair colour. The interaction of modifying genes which control the output of cyanogenetic glucoside in *T. repens* was suggested by Williams (1939). A similar mechanism may also govern enzyme production. It can be assumed on the basis of the present investigation that these modifying factors have also been subjected to natural selection.

It has been shown (Daday, 1954) that in its spread over Europe and the Near East *T. repens* has undergone changes in gene frequencies in response to the January mean temperatures. It may be concluded that a similar response occurs in populations at different altitudes.

5. SUMMARY

1. Populations of *Trifolium repens* from the Central European Alps showed variation in gene frequencies. The dominant glucoside lotaustralin allele (*Ac*) varied from 0·6241 to nil, and the dominant enzyme linamarase allele likewise from 0·5701 to 0·0204. This decrease

was shown to be correlated with the decreasing January temperature at high altitudes.

2. Variation was observed in the amounts of enzyme and glucoside gene products, and this appears to be positively correlated with dominant gene frequencies in the populations.

3. The quantity of enzyme linamarase in plants appears to be governed by modifying genes in a manner similar to the glucoside.

4. Both the differences in gene frequency and gene output are considered to be due to natural selection.

Acknowledgments.—I am greatly indebted to Professor E. T. Jones, Director of the Welsh Plant Breeding Station, for facilities to carry out the investigation, and to Professor P. T. Thomas for his interest in the work. I am also grateful to Mr W. E. Davies, Head of the Clover Breeding Section of the Welsh Plant Breeding Station, for the help he has given, and to Dr J. Caputa, Station Fédérale d'essais et de contrôle de semences, Lausanne (Mont-Calme), Dr A. Kauter, Arbeitsgemeinschaft zur Förderung des Futterbaues, Zürich, and Mr G. A. Toulson, Cornwall Agricultural Executive Committee, Truro, for the seed collections.

I also wish to thank the Director of Schweizerische Meteorologische Zentralanstalt for the temperature data.

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THE CONSEQUENCES OF LOCALISED CHROMOSOME BREAKAGE

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1. INTRODUCTION

CHROMOSOMES can be broken by various chemical reagents in specific regions and at specific times during the mitotic cycle. With 8-ethoxycaffeine (EOC) and maleic hydrazide (MH) breaks and re-arrangements are induced in the resting nucleus and are first observed at the ensuing mitosis (Kihlman and Levan, 1951 ; McLeish, 1952 ; see plate, figs. 1 and 2). The consequences of these limited and regular effects in succeeding cell generations have now been studied in an attempt to answer the following questions :

(i) After breakage at different positions, can the acentric fragments and the micronuclei that they form be classified into recognisable groups ?

(ii) Does the loss or gain of specific parts of nuclei tell us anything about their structure and the elements necessary for their survival ?

(iii) To what extent can the centric and acentric products of primary breakage be used as markers for timing new breakage and reunion in later resting nuclei ?

2. MATERIALS AND METHODS

Seedlings of *Vicia faba* var. Claudia Aquadulce were used in the present experiments. I have already described the methods used in their growth and treatment (1952).

MH treatment.—A dose was selected which, while giving a high breakage frequency, subsequently allowed growth and mitosis to continue so that later cell generations could be studied :

0·5 mM/2 hrs./20° C.

*EOC treatment.**—A dose similar to that employed by Kihlman and Levan (1951) was found suitable in these respects :

8 mM/8 hrs./7° C.

In this case, the plants were kept at 7° C. for 2 days prior to treatment and afterwards were allowed to recover at 20° C.

Preparations.—For metaphase :—

(i) Pre-treatment : Normally, 2 hours in 0·05 per cent. colchicine. In the EOC experiments, breaks in the nucleolar constriction were more easily detected if the chromosomes were excessively contracted so that the acentric fragments became free. Pre-treatment was therefore extended to 4 hours.

* I should like to thank Dr B. Kihlman for his gift of a sample of EOC.

(ii) Fixation : 2 BD overnight.
(iii) Post-fixation : The roots were washed in distilled water and then softened and bleached for 10 mins. in a mixture of ammonium oxalate (sat. aq. soln.) and hydrogen peroxide (20 vol.) 1 : 1 (Dr C. E. Ford, private communication).
(iv) Staining : Feulgen squash method.

For anaphase :—This same technique was used but pre-treatment was omitted.
For studying the positions of acentric fragments and micronuclei :—

(i) Fixation : 2 BD overnight.
(ii) Embedding : 52° C. M.P. paraffin wax.
(iii) Sectioning : at 20 μ.
(iv) Staining : iodine-crystal violet method.

For nucleoli :—A. Sections.

(i) Fixation : 1 : 3 acetic alcohol.
(ii) Embedding : 52° C. M.P. paraffin wax.
(iii) Sectioning : at 6 μ.
(iv) Staining : methyl green-pyronin (Kurnick, 1952).

B. Squashes.

(i) Fixation : formalin-chromic acid overnight (Lewitsky, 1931).
(ii) Staining : Feulgen-light green (Semmens and Bhaduri, 1941).

Measurements.—Outline camera-lucida drawings of nuclei and nucleoli were made at a magnification of ×2520. Their areas were then computed from counts made on a superimposed grid of 1/16 in. squares for nuclei and, to reduce error, 1/32 in. squares for the smaller nucleoli.

Scoring and mapping of breaks.—The symbols of Darlington and La Cour (1945) were used in scoring structural changes. The positions of breaks were mapped by camera-lucida drawings and checked, where possible, by comparisons with the lengths of undamaged homologues.

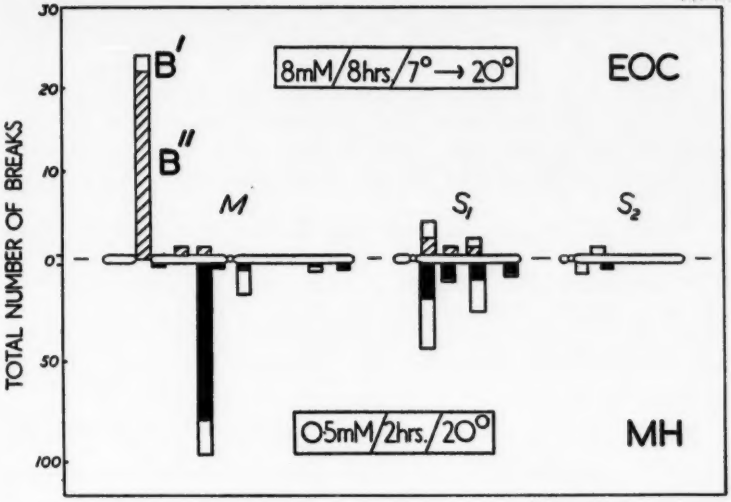
Detection of later cell generations (X_2—X_n).—The progeny of damaged cells were recognised by the occurrence of any one of the following :—

(i) micronuclei ;
(ii) deficiency or translocation of chromosome parts ;
(iii) reproduced acentrics ;
(iv) chromosome reunion (R″) giving dicentric chromosomes—a reunion which very rarely occurs at X_1 (McLeish, 1952).

3. CLASSIFICATION OF ACENTRICS AND MICRONUCLEI

The action of EOC and MH in *Vicia* is to induce limited and localised breakage, EOC chiefly in the nucleolar constriction, MH in the heterochromatin. Its distribution is shown in graphs 1 and 5. Characteristic acentric fragments and micronuclei are thus produced especially by the breakage of the M chromosome.

Acentrics.—Because of the positions of breakage, only four main types of acentric fragment need concern us. They are classified according to the way in which euchromatin, heterochromatin and nucleolar organisers are combined or separated (fig. 1). At X_1 telophase they may be included in main nuclei or lost. If included, all types retain their individuality throughout the ensuing resting stage and reproduce along with the centric chromosomes at X_2 (plate, fig. 7). If lost, they constitute micronuclei of characteristic form.



GRAPH 1.—Comparison of the distribution of breakage segments, in X_1 cells, in the 3 chromosome types M, S_1 and S_2; one day after treatment with EOC (above) and up to and including 5 days after treatment with MH (below). Tabular data not given for EOC. MH data from McLeish (1952), table 4.

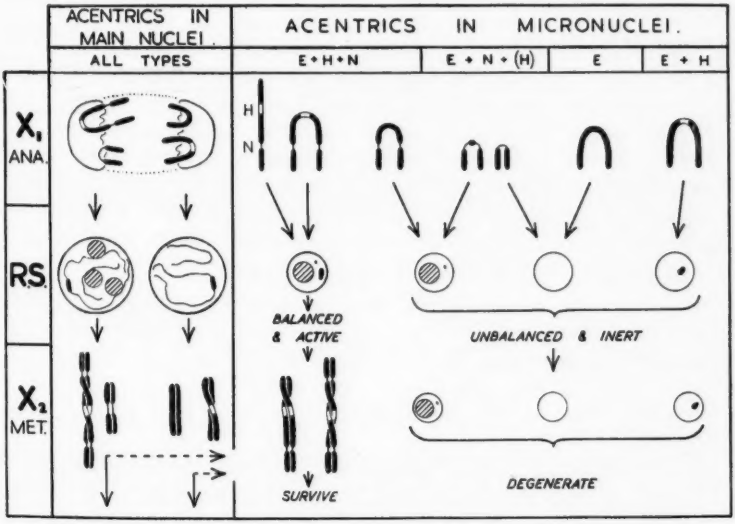


FIG. 1.—Diagram showing how the 4 main groups of acentrics, with various combinations of euchromatin (E), heterochromatin (H), and nucleolar organisers (N), behave when included in main nuclei or micronuclei. The inferred small heterochromatic segment near the nucleolar organiser is not represented. R.S.—resting stage.

Nucleolar organisers are marked by secondary constrictions at mitosis and are thus detectable in acentrics. They organise nucleoli in micronuclei. Heterochromatic segments are, of course, indistinguishable from the euchromatin at mitosis under normal temperature conditions. But they betray their presence in micronuclei by their typical allocyclic behaviour. They are then visible as Feulgen-positive chromocentres (fig. 4, *a-d*; see also Darlington and La Cour, 1940, 1941).

Nearly all acentrics in *Vicia* arise in one of two ways: from B″ SR or B′ R′ (fig. 2). Those arising from B″ SR are *iso-acentrics*. They are double since the arms on each side of the point of reunion are derived from sister chromatids. As we shall see, the extent of such duplication as well as of translocation (B′ R′) in acentrics, influences both the structure and subsequent behaviour of their micronuclei.

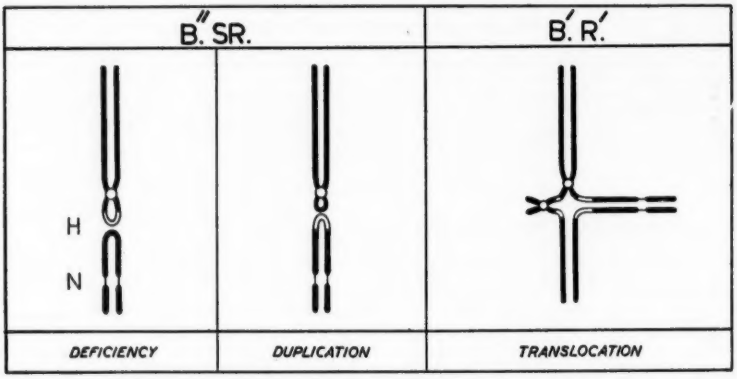


FIG. 2.—Diagram showing how the amount of heterochromatin (H), carried by acentrics with nucleolar organisers (N), can be determined by both the position and the type of breakage and reunion.

Micronuclei.—The contents of micronuclei formed after random X-ray breakage must remain largely unknown unless displayed during a further mitosis. On the other hand, localised chemical breakage can provide a method of segregating known parts of the nucleus as standard acentrics in micronuclei. These, and their complementary but now deficient main nuclei can be examined in some detail.

I have accordingly made measurements of main nuclei, micronuclei and their nucleoli in selected cells of the type shown in fig 4*a* which occur 24 hours after MH treatment. They are daughter cells derived from an X_1 mitosis in which most of the nucleolar arm of one M chromosome had been lost from the nucleus but included in the cell. Their contents can be inferred from the observed breakage

occurring up to 24 hours. This is of low frequency with high localisation in segment 2 (graph 5 ; see also McLeish, 1952, tables 2 and 4). Moreover 94 per cent. of these breaks are B″-s followed by SR to give nucleolar iso-acentrics (fig. 2). Thus the majority of micronuclei selected for measurement contain two sister nucleolar organisers and their main nuclei only one. Nucleoli are organised in both.

At the time of fixation (*i.e.* 24 hours) most micronuclei are less than 12 hours old. This is because, as I previously showed, most breakage appears in mitosis more than 12 hours after treatment. Their size and structure are then accurate indicators of their constitution since any variability due to their survival or degeneration is not yet likely to be apparent.

For comparison, I measured those cells (45·9 per cent.) in untreated roots where the products of the two organisers had fused to give a single nucleolus.

The results of all these measurements are given in graph 2 and are discussed in the next section.

4. THE ORGANISATION OF NUCLEOLI IN *VICIA*

A study of the positions and products of localised breakage can give us an idea of the position of origin of nucleoli and the factors controlling their size.

Position of origin.—The evidence concerning nucleolar origin in *Vicia* is derived from the two types of localised breakage (with EOC and MH) and from observations on X_2 cells.

The positions of breakage with EOC are shown in graph 1. 88·2 per cent. of the resulting acentrics carry varying lengths of the nucleolar constriction (plate, fig. 1) yet only 13·5 per cent. of the total micronuclei have nucleoli (table 1). These micronuclei are approximately the same size as those seen in MH-treated cells (fig. 4*a* ; plate, fig. 3). They are probably formed by acentrics derived from the 6·5 per cent. of breaks occurring between the organiser and the centromere. The production of a nucleolus would then depend not on the constriction region as a whole but on some locus at its proximal end.

The constriction or " SAT zone," observed at mitosis, has often been supposed to be the result of nucleolar growth during the preceding telophase and resting stage. This was suggested by the observations of McClintock (1934) in *Zea Mays* and Navashin (1934) in *Crepis*. The suggestion can be tested in *Vicia* by observing the behaviour of nucleolar acentrics in micronuclei. As we shall see, some of these remain viable ; they reproduce and reappear at X_2 (fig. 6 ; plate, figs. 2 to 5). We then see that the lengths of their nucleolar constrictions are not significantly diminished despite the fact that much smaller nucleoli were formed at the preceding telophase. Possibly the difference in nucleolar size is insufficient to give detectable

TABLE 1

Frequencies of nucleolar and non-nucleolar acentrics and micronuclei at different times after EOC treatment. Scored at X_1 metaphase and anaphase, during the resting stage (R.S.) and at X_n prophase. Nucleolar acentrics are those with nucleolar constrictions

X_1

Time	Cells	Per cent. with acentrics	Total nucleolar	Total non-nucleolar
1 D	Met. 60 Ana. 100	*40·0* *44·0*	25 48	2 8
2 D	Ana. 25	*8·0*	2	...
		Total . . Per cent. .	75 *88·2*	10 *11·8*

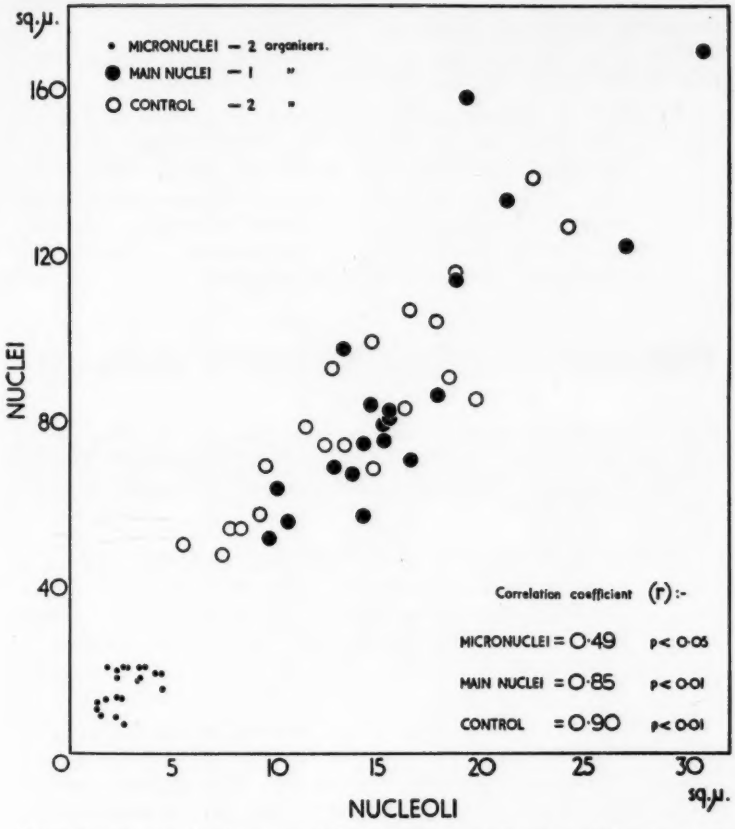
R.S.

Time	Resting cells	Per cent. with micronuclei	Total nucleolar	Total non-nucleolar
2 D	737	*5·4*	5	36
3 D	651	*2·6*	3	16
4 D	635	*2·2*	2	12
		Total . . Per cent. .	10 *13·5*	64 *86·5*

X_n

Time	Prophase cells	Per cent. with micronuclei	Total nucleolar		Total non-nucleolar	
			Active	Inert	Active	Inert
2 D	712	*5·6*	1	3	...	38
3 D	983	*0·61*	...	...	...	6
4 D	947	*1·1*	...	1	...	10
		Total . . Per cent. .	1 *1·7*	4 *6·9*	...	54 *91·4*

differences in constriction length at the ensuing mitosis. On the other hand, the nucleolar constriction of *Vicia* may be a segment of constant length, which, in conjunction with an adjoining proximal locus, forms a compound organiser gene. The constriction could be regarded as a part of the chromosome which is necessary for the proper functioning of the organiser and around which the substances of the nucleolus are secreted (fig. 3).



GRAPH 2.—Showing the linear correlations between nuclear and nucleolar size in the 3 selected classes. Tabular date not given.

Occasionally, in MH-treated diploid cells of *Vicia*, more than two nucleoli appear (fig. 4*c*). Most of these cases probably result from the inclusion in daughter cells at X_1 of extra nucleolar organisers carried by acentrics. Others, as we shall see, appearing 2 days or more after treatment, can be the result of reproduction of such acentrics.

As Heitz (1931) originally suggested, other chromosomes may

have the ability to organise nucleoli. Darlington and Haque (1954) have obtained additional evidence of this from the structure of microcytes at the second meiotic division of *Allium*. In the present experiments with *Vicia* I think such behaviour must be rare for two reasons. First, more than two nucleoli never appear in normal *Vicia* cells; and secondly, in MH-treated cells, the relative frequency of nucleolar and non-nucleolar acentrics at mitosis 24 hours after treatment agrees, approximately, with the relative frequency of their corresponding micronuclei at this time (table 2).

Size.—The following results were obtained from measurements made in the types of selected cells described in the previous section:

(i) There is a significant linear correlation between nuclear and nucleolar size in each of the three classes considered (graph 2; *cf.* Fernandes and Serra, 1944). The scatter may be due to the nuclei being in different physiological stages of interphase since a similar correlation has been observed by Lesher (1951) during increases of chromosome size in salivary gland nuclei of *Drosophila*.

FIG. 3.—Diagram showing the way in which the euchromatin (E), heterochromatin (H) and nucleolar organiser (NO) may be distributed in the nucleolar arm of the M chromosome of *Vicia faba*. C—centromere; NC—nucleolar constriction. The large heterochromatic segment can be revealed by cold treatment. The positions of the two smaller ones are inferred.

(ii) The single nucleolus of the micronucleus is the product of two organisers yet is always smaller than that of its accompanying main nucleus which is the product of one. These latter nucleoli fall into the same range of size as those of controls which are the fusion products of two organisers (graph 2).

(iii) Only 6·6 per cent. of the total micronuclei occurring 24 hours after treatment contain two separate nucleoli in contrast to the 54·1 per cent. of controls. The separate nucleoli are then further reduced in size (fig. 4*b*). Nucleolar fusion here must be due partly to the frequent proximity of sister organisers in iso-acentrics derived from B″ SR (fig. 2), and partly to the restricted internal volume of the micronuclei.

Together, I think these data confirm the general conclusions of McClintock (1934) in showing the size of nucleoli to be influenced by three factors: by competition between their organisers, by the amount of chromosome material which surrounds the organisers, and by the existing physiological condition of their nuclei.

TABLE 2

Frequencies of cells with nucleolar and non-nucleolar acentrics and micronuclei at different times after MH treatment. Scored at X_1 metaphase, during the resting stage (R.S.) and at X_n prophase. Nucleolar acentrics are those with nucleolar constrictions. Reproduced in graph 3.

X_1

Time	Metaphase cells	Per cent. with acentrics	Nucleolar	Per cent.	Non-nucleolar	Per cent.	Both types	Per cent.
1 D	50	*18·0*	5	*55·6*	4	*44·4*	...	...
2 D	200	*31·0*	32	*51·6*	20	*32·3*	10	*16·1*
3 D	200	*23·0*	26	*56·5*	15	*32·6*	5	*10·9*

R.S.

Time	Resting cells	Per cent. with micronuclei	Nucleolar	Per cent.	Non-nucleolar	Per cent.	Both types	Per cent.
1 D	5673	*0·8*	24	*53·3*	20	*44·4*	1	*2·2*
2 D	2025	*10·9*	101	*45·9*	110	*50·0*	9	*4·1*
3 D	2487	*16·7*	180	*43·4*	219	*52·8*	16	*3·8*
4 D	2482	*14·5*	111	*30·7*	238	*65·9*	12	*3·3*
5 D	2231	*8·4*	45	*23·9*	138	*73·4*	5	*2·7*
6 D	1839	*2·8*	21	*40·4*	29	*55·8*	2	*3·8*
7 D	1178	*2·3*	15	*55·5*	11	*40·7*	1	*3·8*

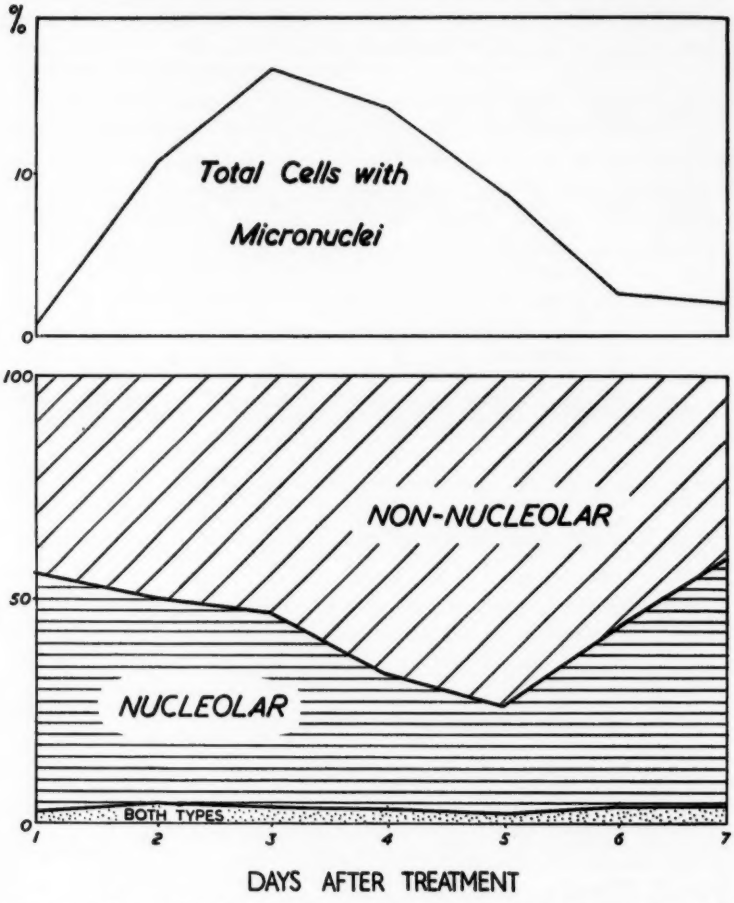
X_n

Time	Prophase cells	Per cent. with micronuclei	Total nucleolar		Total non-nucleolar	
			Active	Inert	Active	Inert
3 D	557	*6·1*	12*	7	...	15
4 D	794	*4·9*	20†	8	...	11
5 D	1023	*1·3*	4‡	1	...	8
		Total . .	36	16	...	34
		Per cent. .	*41·9*	*18·6*	...	*39·5*

* One in advance of main nucleus.
† One retarded slightly.
‡ One cell contained two.

5. THE BEHAVIOUR OF ACENTRICS IN MICRONUCLEI

We have already seen how acentrics can be classified into recognisable groups, each forming characteristic micronuclei. A study of the frequency and behaviour of these micronuclei at various times after their formation shows that they are mostly lost. Of those

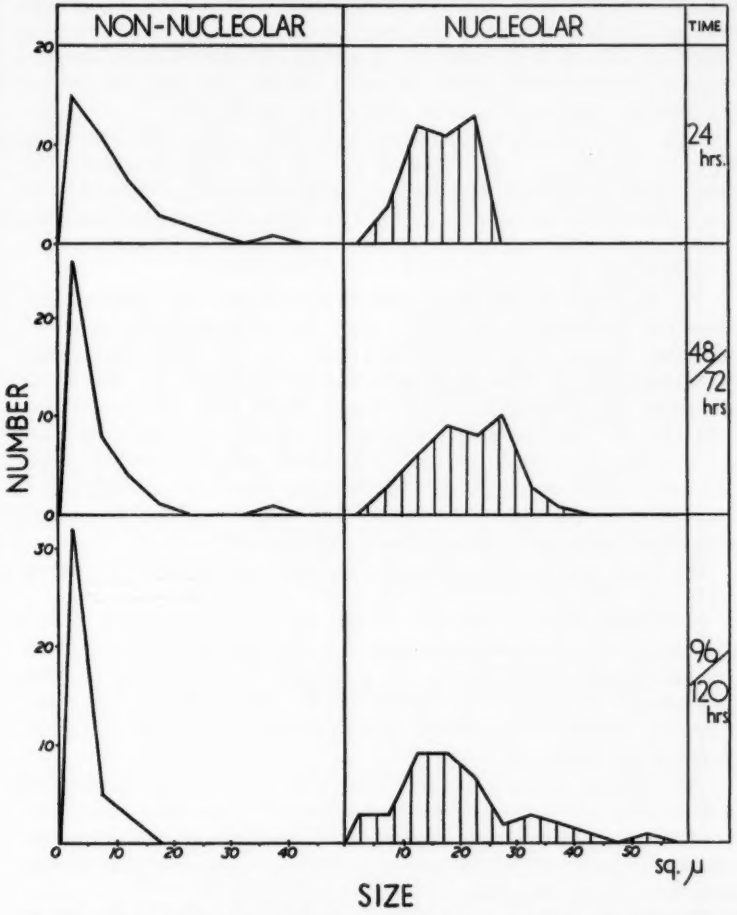


GRAPH 3.—Frequencies of resting cells containing micronuclei at different times after MH treatment. From table 3.

that survive, some appear to be able to play an active part in cell metabolism.

Elimination of micronuclei.—Two main factors operate in eliminating micronuclei from the root meristem. Some degenerate and are absorbed into the cytoplasm or into the main nucleus, while others

are carried away from the apex into differentiated tissue by growth and mitosis. After MH treatment a few new micronuclei continue to arise as a result of deferred breakage. But these are insufficient to interfere with the quantitative estimates of those of primary origin. A small proportion of micronuclei are presumably retained for variable



GRAPH 4.—Comparison of the sizes of nucleolar and non-nucleolar micronuclei at different times after MH treatment. 40 micronuclei of each type were measured for each of the 3 comparisons (the 48 and 72 hrs. and the 96 and 120 hrs. data showed no significant difference and were lumped). Tabular data not given.

times in the meristem by being carried forward in mitosis. The survivors of this proportion must be mostly nucleolar since in contrast to non-nucleolar types, their frequency rises again after 5 days (table 2, graph 3). This conclusion is confirmed in graph 4. Here,

the distinction between the two types is expressed as a size difference. For, while the non-nucleolar micronuclei continually decrease in size with time, the nucleolar types exhibit a greater variability. A spread occurs, representing, as we shall see from other evidence, degeneration in one direction, and in the other, active survival. The degenerating nucleolar micronuclei become pycnotic and hence indistinguishable from non-nucleolar types. This must be the reason for their diverging frequency after 2 days (table 2, graph 3). It also explains the discrepancy between the relative frequency of the two types of acentric and that of their corresponding micronuclei at this time.

One further discrepancy is shown in table 2. After MH treatment, X_1 cells containing both types of acentric are more frequent than resulting daughter cells with both types of micronuclei. This may be due partly to the segregation of the two types into separate daughter cells at X_1 mitosis and partly to the formation of single micronuclei by both types.

The physiology of survival in micronuclei.—During the resting stage following X_1 mitosis (after MH), some excluded nucleolar acentrics evidently remain viable since 69·2 per cent. of their micronuclei become actively synchronised with the main nucleus at subsequent prophases (fig. 4*e* and *f*; plate, fig. 4, table 2).* The remaining 30·8 per cent. may possibly play a minor role in cell metabolism but are evidently incapable of mobilising their proteins, nucleic acids and other components in order to become effective working units. There is consequently no further visible reproduction of their constituent acentrics and they eventually die (fig. 4*d*).

We must now examine the three components which may control the balance between survival and death :—

(i) EUCHROMATIN.—This is present in varying amount in all acentrics, micronuclei and main nuclei. Unlike the nucleolar organisers and heterochromatin it cannot be eliminated from any one of them by localised breakage and hence its relative functions are uncertain. It can, of course, be segregated alone in some micronuclei but these are inert and soon degenerate.

(ii) NUCLEOLAR ORGANISERS.—The active survival of micronuclei is dependant on their containing a nucleolar organiser. La Cour (1952) found this to be true in *Hyacinthus*. In *Vicia* we can determine the relative parts played by nucleolar organisers and heterochromatin since they are to some extent separable by breakage.

(iii) HETEROCHROMATIN.—The amount of heterochromatin which is carried into the micronucleus is known to be variable. It depends directly on the position of breakage and on the type of reunion (figs. 1 and 2).

All nucleolar micronuclei are formed by acentrics derived entirely or in part from breakage in the nucleolar arm of the M chromosome. The combined evidence from cold treatment and breakage with

* Acentrics derived from EOC breakage are rarely viable (see table 1).

MH has shown that this arm carries one of the largest blocks of heterochromatin in the *Vicia* nucleus—corresponding with breakage segment *2*—and possibly two smaller blocks corresponding with breakage segments *1* and *3* (fig. 3). There may be additional smaller blocks which have so far eluded detection since numerous tiny chromocentres can occasionally be resolved in the micronuclei (fig. 4*d*).

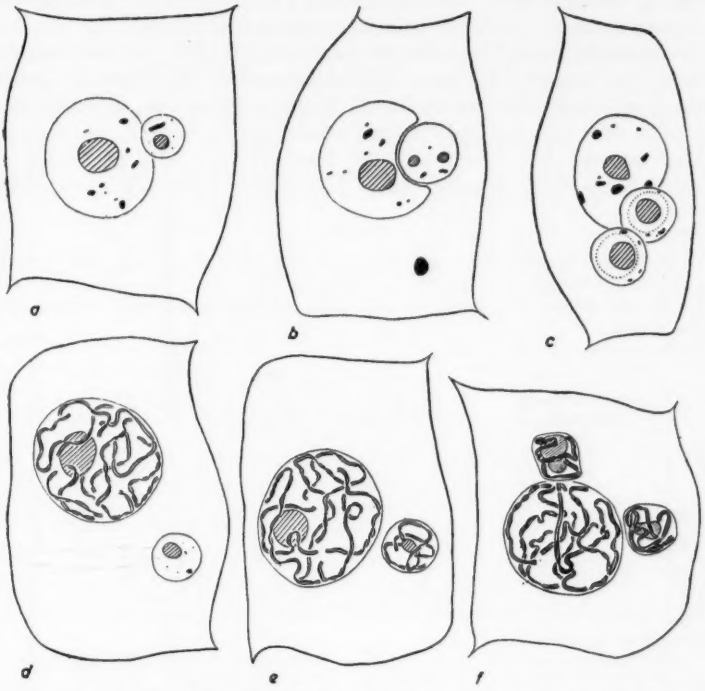


FIG. 4.—(*a*) to (*f*). Root cells of *Vicia faba* containing micronuclei (m.n.) at different times after MH treatment.

(*a*) 24 hrs. : main nucleus and m.n. containing heterochromatin (black) and nucleoli (hatched).
(*b*) 48 hrs. : nucleolar m.n. partially enclosed by main nucleus. Small pycnotic m.n. degenerating in cytoplasm.
(*c*) 72 hrs. : 2 nucleolar m.n. A third nucleolus in the main nucleus.
(*d*) 72 hrs. : Active main nucleus at prophase. Inert nucleolar m.n. deficient in heterochromatin.
(*e*) 72 hrs. : Main nucleus and nucleolar m.n. synchronised at prophase.
(*f*) 96 hrs. : Main nucleus (deficient in nucleolar organisers) synchronised with 2 nucleolar m.n. ×1300.

Even allowing for the possibility of fusion, the largest block must form one of the most prominent chromocentres in the resting nucleus. Yet, during resting stage 24 hours after treatment, only 44·4 per cent. of nucleolar micronuclei contained chromocentres which appeared to be of comparable size.

This may represent the proportion which remains viable. For, later on, of the 30·8 per cent. which were inert at X_n prophase, only 2 contained such large chromocentres; 10 had small to medium-sized chromocentres and 4 had none at all. Evidently, heterochromatin was the balancing factor for viability.

However, one inert micronucleus contained a nucleolus, 3 large and 3 medium-sized chromocentres. It apparently had all the requirements for activity—adequate heterochromatin and at least one nucleolar organiser—and yet was inert. Possibly, a non-nucleolar acentric, carrying the extra heterochromatin, had been included along with the nucleolar acentric. If this was the case, it would then appear that for activity, the nucleolar acentric must carry its own requirements of heterochromatin. The presence of a small amount near the nucleolar organiser which I previously inferred from breakage

TABLE 3

Localisation of the total breakage and reunion occurring up to 28 days after MH treatment in cell generations after X_1. High R′ values are due to the reunion of broken ends from X_1 with deferred B′-s (see fig. 8). As at X_1, unreunited ends are rare. Reproduced in graph 5

Breakage segments (centrics)	B″	SR		B′	R′
		C_1	C_0		
2	5	*5*	*5*	2	4
7	...	...	...	2	2
8	1	*1*	*1*	2	2
10	1	*1*	*1*	4	6
11	...	...	...	1	2
14	1	*1*	*1*	1	...
Acentrics : all nucleolar					
Median	9	8	8	2	4
Sub-median	1	1	1	...	...

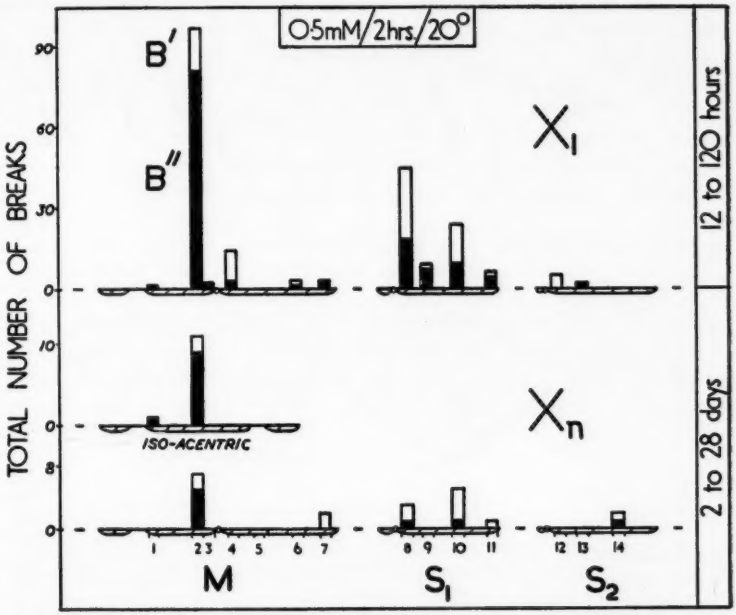
in segment *1* is apparently not enough. Although short acentrics derived from breakage in this segment can occasionally reproduce, I think they do so only after inclusion in the main nuclei.

Supporting evidence that heterochromatin is the decisive factor for the survival of nucleolar micronuclei, comes from the localisation and frequency of deferred breakage. This, as we shall see, occurs at X_2 and X_3 following MH treatment. Breakage in these cells is more frequent in the middle of the nucleolar iso-acentric than in segment *2* of the remaining normal M chromosomes (table 3, graph 5, fig. 5*b*). Breakage frequency seems to be roughly proportional to the size of heterochromatic segments as it is at X_1 (McLeish, 1952). Thus it follows that the segment in these nucleolar iso-acentrics must be as large, if not larger, than the segment in the M chromosomes.

It may be lengthened as a result of duplication by SR (fig. 2). This would also account for the long rod-like chromocentres which can sometimes be seen in micronuclei (fig. 4*a*).

These cells are, of course, a selected sample. The acentrics can reproduce because of their large content of heterochromatin, yet it is through this heterochromatin that they are more susceptible to breakage.

From their study of the B chromosomes in *Sorghum*, Darlington and Thomas (1941) have suggested that the effect of heterochromatin is to stimulate nuclear and hence cell division. Further, Caspersson *et al.* (see Caspersson, 1950) have shown that heterochromatin and



GRAPH 5.—Distribution of the segments (numbered *1* to *14* in the 3 chromosome types M, S_1 and S_2) in which primary breaks (at X_1) and deferred breaks (at X_n) occur after MH treatment. The change in the relative breakage frequencies in nucleolar and non-nucleolar chromosome arms in X_n cells is not significant. From table 3 and McLeish (1952), table 4.

nucleoli are chemically inter-related ; they appear to share similar functions in the protein and nucleic acid metabolism of the cell. The present results confirm the importance of their physiological relationship for chromosome reproduction and the working existence of the nucleus.

Nuclear co-operation.—The studies of Barber (1941) in *Uvularia* have shown how complementary and physiologically active resting nuclei can co-operate to make a balanced system in the cell.

In *Vicia* the close association of a main nucleus with a nucleolar micronucleus often gives us an indication of their co-operation and of their impending synchronisation in mitosis. Non-nucleolar types seem never to be associated or synchronised with main nuclei in this way.

In 14·5 per cent. of cases, the nucleolar micronucleus is partially enveloped by the main nucleus (fig. 4*b*). Yet the two remain distinct with a clear dividing zone and do not fuse. This must be due to the fact that nuclei are bounded by real membranes and not merely

FIG. 5 (*a*) to (*d*).—Metaphase cells derived from the X_1 generation following MH treatment. Showing fragment chromosomes (black) and their homologues (stippled where recognisable).

(*a*) Cell deficient in the nucleolar arm of one M chromosome. The other M and two S_1 chromosomes show partial breakage.
(*b*) X_2 cell with reproduced nucleolar iso-centric with deferred B″+SR.
(*c*) Deferred breakage and reunion, involving an M chromosome and a nucleolar acentric, giving a triradial.
(*d*) An $S_1 : S_2$ triradial. No fragment present. ×1250.

surfaces (Callan and Tomlin, 1950; Bovey—see Wilkins, 1951). Dowrick (1954) has seen a similar situation in pollen grains of the diploid pear, Beurré Bedford. They contain groups of nuclei which remain closely associated but never fuse until mitosis when the membranes have gone.

In *Vicia* the close nature of the relationship between main nucleus and micronucleus is shown by the way they react to the loss or gain of nucleolar organisers. Evidently, the loss of one organiser from the nucleus and the cell does not impede mitosis since I have observed

29 metaphases deficient in the nucleolar arm of one M chromosome (fig. 5*a*). But if both organisers are lost, it seems that no further mitosis can occur. If, however, both organisers are lost from the nucleus but included in the cell in micronuclei, a balanced system can result. Then the main nucleus is helped along by its two accessory nuclei and all three co-operate during the resting stage to achieve synchronisation at mitosis (fig. 4*f*).

This raises a further question. Why is it that a balanced micronucleus can help a deficient main nucleus and yet a balanced main nucleus cannot help a deficient micronucleus? The answer may lie in the fact that these micronuclei differ from main nuclei in one constant respect: they never contain centromeres.

We know that apart from its well-known function on the mitotic spindle, the centromere can be distinguished by its earlier behaviour inside the nucleus. It is known to influence the localisation of X-ray and ultra-violet radiation breakage as well as the frequency of sister reunion (Sax and Mather, 1939; Swanson, 1942; Darlington and Upcott, 1941; Darlington and La Cour, 1945). In *Scilla*, Rees (1952) has shown how it interferes with X-ray-induced errors of chromosome separation at anaphase.

In the absence of nucleolar organisers and detectable heterochromatin, centric chromosomes can form viable micronuclei in *Uvularia* (Barber, loc. cit.). Here, as in the case just described in *Vicia*, nucleolar organisers were present in the accompanying nucleus.

Thus the absence of heterochromatin or nucleolar organisers or both reveals that the centromere can play a part in maintaining the viability of a nucleus. This nucleus is then capable of co-operating in development with one or more complementary nuclei.

6. REPRODUCING ACENTRICS AS CYTOLOGICAL MARKERS

After MH treatment, some acentric fragments survive and reproduce through successive mitoses in viable micronuclei as can all types by inclusion in main nuclei. They all provide markers for timing the deferred breakage and reunion which continues to arise from the persistent effects of initial treatment.

Nucleolar acentrics were originally reported as reproducing in *Vicia* by Levan and Lotfy (1951). They occur frequently in the present experiments and provide the most reliable markers (plate, fig. 6). Occasionally they can be studied in X_2 and X_3 prophase micronuclei. They appear to be able to reproduce through two successive mitoses; an original acentric chromatid gives four chromatids in two pairs (fig. 6). The paired chromatids often show relational coiling. At X_2 anaphase they rarely fall far apart and are both included in one of the daughter cells. Thus a further reproduction is an accurate indication of X_3.

I have no evidence of their ability to undergo a further reproduction

at X_4. By this time, the cells may have become unbalanced. Many will also have passed into differentiated tissue. In either case, they will not undergo mitosis again.

7. DEFERRED BREAKAGE

Initial treatment with MH causes breakage at X_1 and in succeeding cell generations. It occurs at X_2 and X_3 with similar type and distribution as at X_1 but with diminishing frequency (tables 3 and 4; graph 5, fig. 6). It occurs in hitherto undamaged chromosomes, in centric fragment chromosomes and in acentric chromosomes which have survived either by inclusion in main nuclei or through viable

TABLE 4

Frequencies of cells in secondary lateral roots of Vicia faba, *showing primary and deferred breaks at different times after MH treatment. Some cells can be timed in relation to the initial breakage (see text). The history of normal cells cannot be determined since they are unmarked*

Time	Cells	Normal	Broken						
			X_1	X_2		X_3		Untimed X_n	
				Total	Rebroken	Total	Rebroken	Total	Rebroken
2 D	200	125	72	3	...	...	...	...	...
3 D	315	219	61	15	3	...	...	20	1
4 D	266	164	35	34	7	4	2	29	4
5 D	63	46	4	8	1	...	...	5	2
6 D	65	50	2	5	1	2	1	6	2
10 D	28	19	1	3	1	...	...	5	1
19 D	18	12	...	3	2	1	...	2	1
28 D	30	24	2	2	...	...	...	2	...

micronuclei (figs. 5 and 6). Furthermore, X_1 and X_2 breakage reappears in newly formed tertiary roots 28 days after treatment.

Deferred breakage may be the later expression of the partial breakage seen at X_1. Particularly if, as Darlington (1950) suggests, the chromosome has a multiple fibre structure. For breakage occurring in a few of these sub-units would not become effective in the whole chromosome until they had been sorted out in successive mitoses.

An alternative and more likely explanation is that the reagent persists in the cells for a long period, where it continues to induce its characteristic action in the chromosomes. In view of its molecular structure, which has been discussed by Kihlman (1951), Loveless (1952) and others, it could conceivably be incorporated in the cell in some way to form a substitution nucleic acid precursor.

A further indication of its continued presence is its permanent effect on the size of the meristematic zone. This is gradually reduced

in length during the first 3 days after treatment and often remains so for at least 28 days. Within this zone, however, mitosis continues at an approximately normal level. The effect on mitosis is probably,

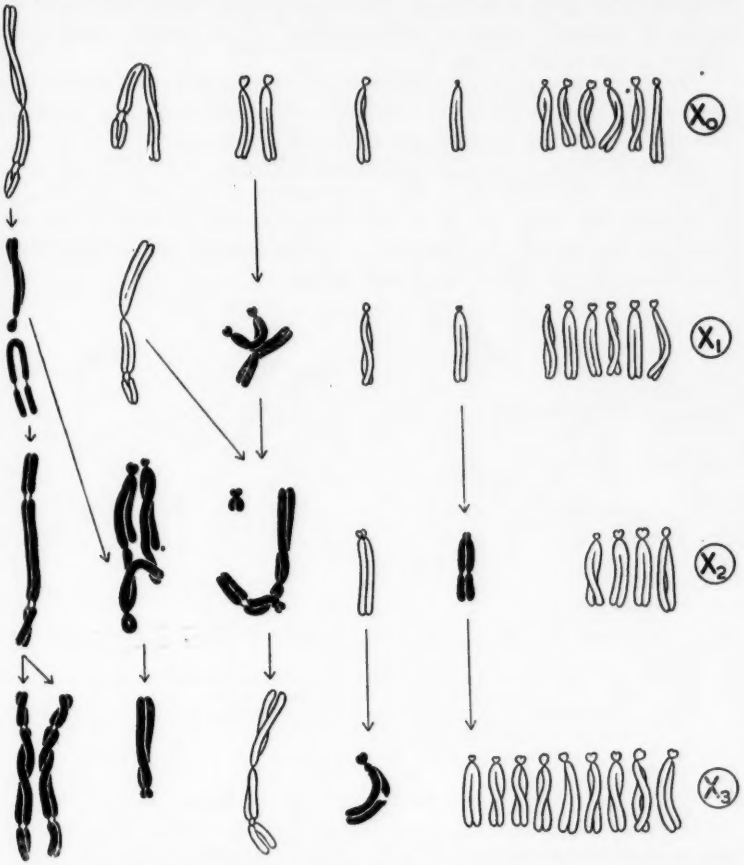


FIG. 6.—Metaphase chromosomes observed in a normal cell (X_0) and in 3 consecutive cell generations (X_1 to X_3) after MH treatment. Illustrating some of the possible ways in which configurations formed at one mitosis can influence those formed at the next. X_2 and X_3 are timed in relation to X_1 by the number of reproductions of the nucleolar iso-acentric (left).

X_1, 48 hrs.: B″ SR in segment 2 giving a nucleolar iso-acentric. $S_1 : S_1$ interchange forming a C'_2.

X_2, 96 hrs.: fragment chromosomes from X_1 reunited with deferred breaks. Partial break in an S_2 chromosome.

X_3, 96 hrs.: Deferred B′ in an S_1 chromosome. ×1250.

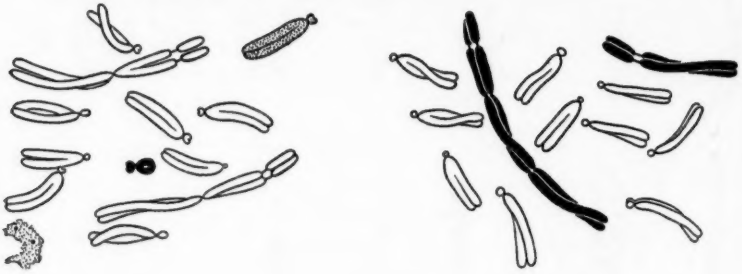
as I pointed out previously, something different from the chromosome breaking effect. It could possibly be correlated with the findings of Leopold and Klein (1951) that MH is an anti-auxin.

8. SECONDARY REUNION

The chief consequences of X_1 anaphase separation, after MH treatment, are shown in fig. 8. Dicentric chromatid bridges resulting from SR or R′ break at random. The two daughter nuclei then receive equal or unequal fragment chromosomes whose broken ends can behave in one of three ways :

(i) They can undergo R″ with other broken ends to give dicentrics (fig. 7*b*). This occurs in a restricted period of movement during telophase which has been defined by La Cour (1952).

(ii) If R″ does not occur before the initiation of the X_2 mitosis, the broken ends then have the opportunity to undergo secondary or delayed SR (fig. 7*a*) as in the breakage-fusion-bridge cycle of McClintock (1941). In *Vicia* after MH treatment, as in *Hyacinthus* after X-raying (La Cour, 1952), this is rare.



a *b*

FIG. 7 (*a*) and (*b*).—Secondary reunion in X_2 metaphase cells of *Vicia* 96 hrs. after MH treatment.

(*a*) SR in an S_1 centric fragment ; remainder of chromosome present as a micronucleus. Possible undamaged S_1 homologue stippled.

(*b*) C_2″ derived from non-homologous R″ between the broken ends of two M fragment chromosomes carried through from X_1. Nucleolar Co″ formed by reproduction of an original Co′. ×1250.

(iii) Finally, the residue of broken ends failing to undergo either R″ or SR, remain open for a sufficient time to reunite with centric and acentric fragments which have suffered deferred breakage. Such reunion of new and old ends accounts for the common occurrence, at X_2, of triradials (fig. 5*c* and *d*).

This sequence of events shows SR to be a different type of reunion from either R″ or R′. For, although the separate sister chromatid ends remain capable of reunion after the split, they do not seem to reunite with one another. Thus, it is easier to look upon SR as resulting earlier from failure of chromosome reproduction rather than later from a real reunion of sister chromatids.

An accurate quantitative assessment of these various types of secondary reunion is impossible for the following reasons :

(i) Dicentric chromosomes seen at X_2 need not be the result of

R″. They can also arise by the subsequent duplication of complete dicentric *chromatids* which passed complete to one pole at X_1 (fig. 8). (ii) The absence of SR in fragment chromosomes at X_2 is not necessarily an indication of its failure. Such small chromosomes can arise from chromatid translocation at X_1. Their breakage and reunion points are interstitial and consequently hidden and sealed against further reunion. (iii) In a somewhat similar way, the high frequency of

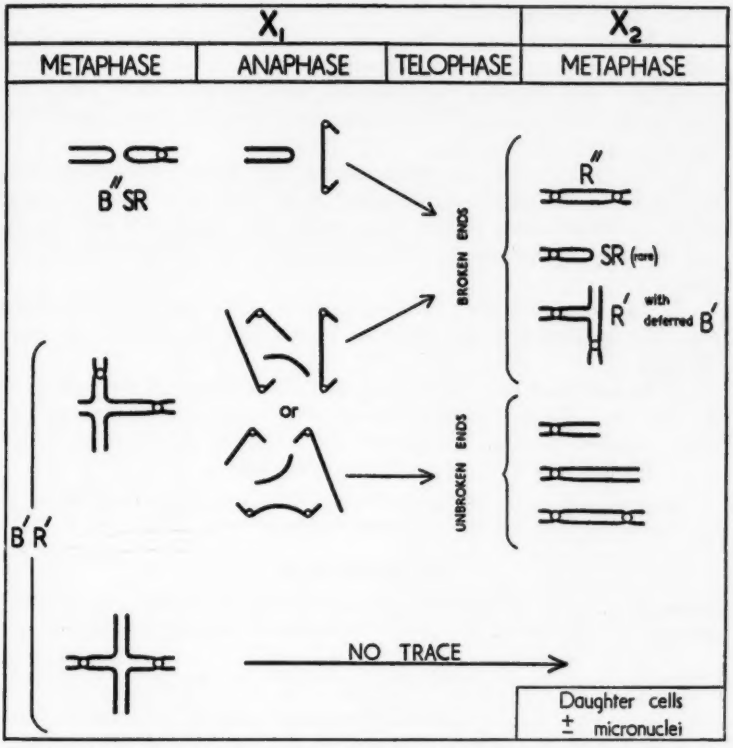


FIG. 8.—Typical X_1 metaphase configurations seen after MH treatment and the chief consequences of their separations at anaphase. Derived X_2 daughter cells may or may not contain micronuclei.

reunion in acentrics at X_1 leaves few with broken ends (McLeish, 1952). Thus even if they are included in main nuclei, they are seldom involved in secondary reunion unless re-broken.

9. SUMMARY

1. Treatments with maleic hydrazide (MH) and 8-ethoxycaffeine (EOC) lead to specific and different patterns of chromosome breakage and reunion in *Vicia faba*. Standard types of centric and acentric fragments therefore enter into the main nuclei and the micronuclei.

2. Acentrics and micronuclei can thus be classified into four types according to their combinations of euchromatin, heterochromatin and nucleolar organisers.

3. Nucleoli are organised in some micronuclei. As in the main nuclei, they appear to be formed on a specific chromosome segment, by an organiser at the *proximal* end of the secondary constriction. Their size is influenced by competition between their organisers and by the size of the nuclei.

4. Viable micronuclei have to carry both nucleolar organisers and heterochromatin. They co-operate with main nuclei during interphase and become synchronised with them during prophase. The nuclei, which have definite membranes, are often closely associated, but they rarely, if ever, fuse.

5. When included in main nuclei, all types of acentric can reproduce in successive mitoses (X_2, X_3). Nucleolar types can also do so in micronuclei. They thus mark the number of the mitosis and show when deferred breakage and secondary reunion are taking place.

6. The regular pattern of breakage seen at X_1 after MH treatment can thus be shown to be repeated in X_2 and X_3 cells for at least 28 days.

7. Broken ends in centrics and acentrics are carried through from X_1 and X_2. They can then reunite with contemporary, as well as with deferred, breaks. Moreover the reunions are of new kinds: at X_1-SR but rare R″; at X_2-R″, but rare SR.

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Plate

FIG. 1.—X_1 anaphase after EOC. Breakage and reunion (probably B″SR) has occurred in the nucleolar constriction, part of which is now visible in the centre of the C_2' bridge and part in the centre of the Co′.

FIGS. 2 to 5.—Reproduction of a balanced acentric after MH breakage.

2. X_1 anaphase. Breakage and sister reunion (B″SR) in segment *2* has given a nucleolar iso-acentric and a C_2'. The undamaged sister M chromosomes have passed to the poles.

3. Daughter cell arising from a mitosis as shown in fig. 2. Nucleoli organised by the undamaged M chromosome in the main nucleus and by the nucleolar iso-acentric in the micronucleus.

4. Synchronisation of main nucleus and micronucleus at X_2 prophase.

5. Early anaphase of X_2. The original Co′ is now a Co″.

FIGS. 6 and 7.—X_2 metaphases after MH.

FIG. 6.—B″SR probably occurred in segment *2* of both M chromosomes at X_1. Both the resulting nucleolar iso-acentrics have reproduced (at 3 and 5 o'clock); one shows a median deferred break. The centric fragments have undergone R″ to give a C_2''.

FIG. 7.—A non-nucleolar acentric (9 o'clock) derived from breakage in an S_1 chromosome at X_1 has reproduced by inclusion in a main nucleus. The centric fragment is lying in the centre of the cell.

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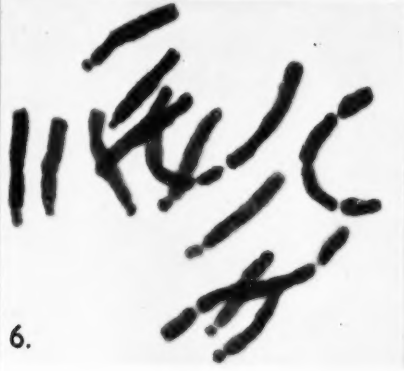
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FERTILITY IN *LILIUM* HYBRIDS

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1. INTRODUCTION

FERTILITY, as measured by seed setting after self or cross pollination, depends on pollen fertility, embryo-sac fertility, success of fertilisation and normality of embryo and endosperm development. Fertility can be reduced by abnormalities in any of these stages and in structural hybrids both genotypic and segregational sterility operates. Segregation in structural hybrids leads to both genic and numerical unbalance

TABLE 1

Percentage of Lilium *hybrids*

Hybrid	Generation	♀ Parent	♂ Parent
Marhan	F_1	*Martagon album*	*Hansonii*
Dalhansoni	F_1	*Martagon dalmaticum*	,,
Stenographer group hybrids—			
Lyla McCann	Multiple hybrids	*Davidi Willmottiae*	Unnamed seedling of *L. dauricum* × *L. maculatum*
Lilian Cummings			
Edna Kean			
Phyllis Cox			
Coronation	Multiple hybrids	Unnamed " Stenographer Lily "	Open pollinated

in the gametes. Unbalanced gametes cannot survive and fertility is reduced. Individually viable gametes can produce unbalanced combinations, resulting in sterility in the zygote.

The development of squash techniques allows us to examine the male and female gametes and also the developing seeds. It offers us an opportunity of re-examining the abnormalities reducing fertility in structural hybrids.

2. MATERIALS AND METHODS

Two F_1 hybrids, *Marhan* and *Dalhansoni*, and several multiple hybrids were used in these studies. Parentages are given in table 1 (Woodcock and Stearn, 1950). A number of species including both self-fertile and self-sterile forms were used throughout as controls.

Meiosis in pollen mother cells (PMC) was studied in smears stained with Feulgen reagent (hydrolysis N/1 HCl, 60° C., 6 mins.) or aceto-carmine squashes made after fixation in methyl alcohol or acetic alcohol (1 : 3).

Embryo-sacs and endosperms were dissected and squashed in 45 per cent. acetic acid after fixation in Carnoy fluid (6 : 3 : 1) and staining with Feulgen reagent (hydrolysis 10 mins.).

Pollen tube growth in styles and pollen fertility were measured after staining with 0·4 per cent. cotton blue in lacto-phenol.

For fertilisation studies ovules were embedded in wax, sections cut 40 μ thick and stained with crystal violet.

3. DEVELOPMENT OF POLLEN

Meiosis in pollen mother cells.—The species had high chiasma frequencies and complete pairing at M I. The hybrids had reduced chiasma frequency (table 2) which resulted in univalent formation (fig. 1).

TABLE 2

Metaphase pairing and chiasma frequency in PMC of Lilium *species and hybrids (12 cells in each)*

	Cells	Metaphase pairing				I per cell	Chiasma frequency	
		12^{II}	11^{II}	10^{II}	9^{II}		Total Xta	Xta p. pot. biv.
SPECIES								
L. candidum . .	50	49	1	...	...	0·02	453	*3·15*
L. Davidi . .	50	50	...	...	...	...	478	*3·32*
L. Hansonii . .	50	50	...	...	...	...	480	*3·33*
L. Henryi . .	50	50	...	...	...	...	496	*3·44*
L. pardalinum .	50	50	...	...	...	...	530	*3·68*
L. regale . .	50	50	...	...	...	...	556	*3·86*
FIRST CROSSES								
Marhan . .	50	33	16	1	...	0·72	382	*2·56*
Dalhansoni . .	50	43	7	...	...	0·28	401	*2·78*
MULTIPLE HYBRIDS								
Lyla McCann .	50	25	24	...	1	1·08	299	*2·01*
Lilian Cummings .	50	40	9	1	...	0·44	361	*2·51*
Edna Kean . .	50	36	14	...	...	0·56	383	*2·66*
Phyllis Cox . .	50	46	4	...	...	0·16	433	*3·01*
Coronation . .	50	29	17	3	1	1·04	384	*2·67*

^{II} = Bivalent. ^{I} = Univalent.

Crossing-over in inverted segments in structural hybrids results in bridges and fragments at first or second anaphase (Richardson, 1936). The inversion crossing-over frequency is a measure of hybridity with respect to inverted segments, but the reduced chiasma frequency in the hybrids tends to mask the full expression of these changes. A more accurate estimate of the hybridity is given by the coefficient of hybridity which compensates for the reduced crossing-over in the inverted segments (table 3).

Plotting the chiasma frequency against the inversion crossing-over frequency or the coefficient of hybridity (fig. 2) clearly indicates the different orders of structural hybridity in the different hybrids. The " stenographer group " hybrids all have a similar level of structural

hybridity. The F_1, *Marhan*, resulting from different parents, has much increased hybridity. The open pollinated derivative from the "stenographer group", Coronation, is clearly not a result of selfing within the group for its hybridity is increased. This is borne out by the unusual flower colour of this plant, yellow with purplish-maroon spots, compared with the orange-red flowers of the stenographer group hybrids.

A further indication of structural hybridity is the segregation for a factor or factors controlling the rate of cell division. This is observed as failure of synchronisation of sister cells at second division of meiosis (table 3). This characteristic may be useful in detecting hybrids

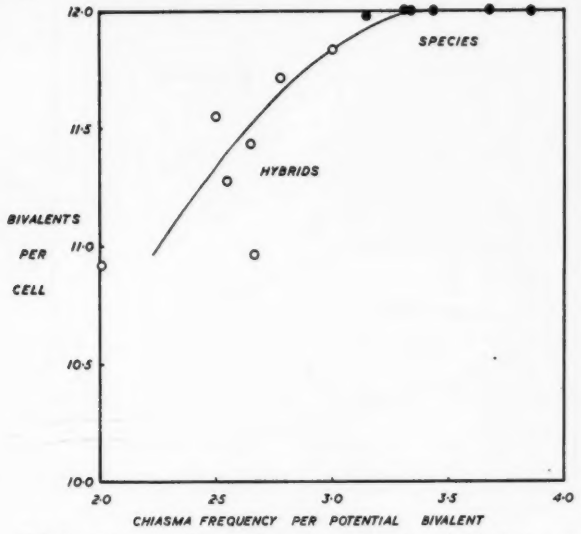

FIG. 1.—Relation between chiasma frequency per potential bivalent and metaphase pairing in PMC of *Lilium* species (closed circles) and hybrids (open circles).

where inversion crossing-over is absent or very rare. A further abnormality which reduces pollen fertility is spontaneous chromosome breakage in PMC. This was to be inferred in two ways :—(i) in *L. candidum* and Lilian Cummings fragments occurred at M I and M II accompanied by greatly reduced chiasma frequency at M I. (ii) In several of the hybrids there was occasionally sister reunion of unbroken ends. This gives rise to anaphase bridges without accompanying fragments. Mechanical breakage of the bridges results in chromosome breakage.

Loss of lagging univalents and breaking of anaphase bridges after inversion crossing-over will result in pollen grains deficient for chromosomes or parts of chromosomes. Such deficient nuclei, except in rare cases such as in *Hyacinthus* (Brock, unp.) are expected to be inviable. Do they in fact reduce pollen fertility ?

Pollen fertility.—Considering the species and the hybrids I find a relationship between the chiasma frequency and the pollen fertility (fig. 3). In the hybrids pollen fertility is more closely related to the univalents per cell plus the inversion crossing-over frequency per cell (fig. 4) for this takes into account the loss due to inversions as well as that due to lagging univalents.

Thus segregation is the main factor reducing pollen fertility. The reduction in fertility was in no case complete (table 7). As a large

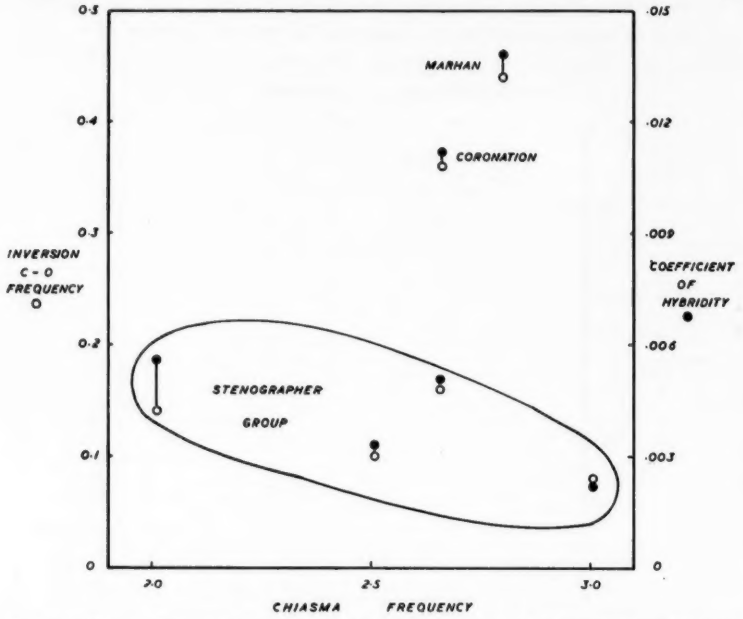

FIG. 2.—Chiasma frequency per potential bivalent plotted against inversion crossing-over frequency and coefficient of hybridity in PMC of *Lilium* hybrids.

surplus of pollen is produced this pollen sterility probably has little direct effect on the overall fertility of the plants.

4. EMBRYO-SAC DEVELOPMENT

Normal development.—The normal development of the embryo-sac in *Lilium* is illustrated in fig. 5 and plate 1. Mitoses are synchronised and no cell walls are formed until the embryo-sac is fully developed. After meiosis one of the haploid nuclei migrates from the micropylar end to the chalazal end of the embryo-sac. At the next mitosis the remaining micropylar nucleus divides, at the same time the three chalazal nuclei form a common spindle and produce two triploid nuclei. One more mitosis produces four haploid and four triploid nuclei. The haploid nuclei form the egg, two synergids and one

TABLE 3

Inversion crossing-over frequency, coefficient of hybridity in regard to inversions and synchronisation of sister cells at second division in PMC of Lilium *species and hybrids*

	Inversion c.-o.								
	PMC	A I	b+f	PMC	A II b+f	Inv. c.-o. frequency	Coeff. of hybridity	PMC	Not synchronised at M II or A II
		Single	Double						
SPECIES									
L. candidum	100	...	...	100	...	...	...	100	None
L. Davidi	50	...	...	50	...	...	...	100	
L. Hansonii	100	...	...	100	...	...	...	100	
L. Henryi	40	...	...	40	...	...	...	50	
L. pardalinum	50	...	...	50	...	...	...	100	
L. regale	100	...	...	100	...	...	...	100	
L. Szovitsianum	...	...	...	100	...	...	...	100	
F_1's									
Marhan	50	9	1	50	7	0·44	0·0138	50	10 per cent.
Dalhansoni	...	...	...	...	...	...	...	...	...
MULTIPLE HYBRIDS									
Lyla McCann	100	9	...	100	5	0·14	0·0056	100	14 per cent.
Lilian Cummings	100	5	...	100	5	0·10	0·0033	100	18 ,,
Edna Kean	100	9	...	100	7	0·16	0·0050	100	11 ,,
Phyllis Cox	100	4	...	100	4	0·08	0·0022	100	8 ,,
Coronation	50	10	...	50	8	0·36	0·0112	100	30 ,,

Note.—Inversion crossing-over frequency calculated by adding together the frequency of bridges at first and second division per mother cell, counting double first division bridges as six (Darlington, 1937, p. 271).

Coefficient of hybridity in regard to inversion calculated by dividing inversion crossing-over frequency by chiasma frequency per cell.

TABLE 4

Metaphase pairing and chiasma frequency in embryo-sacs of Lilium *species and hybrids*

	Metaphase pairing						Chiasma frequency		
	Cells	12^{II}	11^{II}	10^{II}	9^{II}	Mean univalent per cell	Cells	Total Xta	Xta p. pot. biv.
SPECIES									
L. Hansonii	2	2	...	...	...	...	1	49	4·08
L. regale	28	28	...	...	...	...	2	94	3·92
F_1's									
Marhan	3	1	1	1	...	2·0	3	72	2·00
Dalhansoni	10	8	2	...	...	0·4	4	129	2·69
MULTIPLE HYBRIDS									
Lyla McCann	5	2	1	2	...	2·0	3	70	1·94
Lilian Cummings	5	3	1	1	...	1·2	1	33	2·75
Edna Kean	15	9	4	1	1	1·2	4	97	2·02
Phyllis Cox	11	9	2	...	...	0·4	2	69	2·87

Note.—Two cells of *L. candidum* and 5 cells of *L. pardalinum* each had 12^{II}s.

polar nucleus. The triploid nuclei form the other polar nucleus and the antipodals.

Abnormalities occurred frequently in hybrids and rarely in the species. Some of the abnormalities are illustrated in plate 2. They are of two kinds, chromosomal abnormalities and errors of synchronisation or differentiation.

Chromosome abnormalities.—The chiasma frequency in the embryo-sac mother cells was similar to that in the PMC's (fig. 6) as was also

TABLE 5

Abnormal chromosome distribution in individual embryo-sacs of Lilium *species and hybrids*

Stage	Hybrid or species	Chromosome distribution	Cause
A II ($x : x$, $x : x$)	Lyla McCann	10 : 10, 13 : 13 +dicentric bridge	10 : 14 distribution from univalents at M I ; b+f inv. c.-o. (pl. 2, fig. 3)
	Marhan	48	Restitution of all four haploid nuclei
M III ($x : 3x$)	*L. regale*	11 : 37	11 : 13 distribution from univalents at M I
	Dalhansoni	11 : 37	11 : 13 distribution from univalents at M I
	Marhan	10 : 38	10 : 14 distribution from univalents at M I
	Lyla McCann	13 : 35	13 : 11 distribution from univalents at M I
	Lilian Cummings	12 : 34 +dicentric	A I 12 : 12 A II 12 : 12, 11 : 13. Dicentric bridge carried complete (pl. 2, fig. 6)
	Dalhansoni	23 : 23+active micronuclei	Univalent excluded as micronucleus at A I or A II Abnormal fusion at M III

found by Frankel *et al* (1940). As with the PMC's their reduced chiasma frequency results in univalents at M I. No misdivision of the centromere was observed but random movement of univalents at first or second division of meiosis results in irregular distribution of chromosomes (table 5) or the formation of micronuclei (table 6).

Inversion crossing-over results in bridges and fragments at first or second division. Bridges may break or be carried complete to one nucleus, or persist as interphase connections. When the bridge is carried complete to one nucleus (plate 2, fig. 1) it forms a dicentric chromosome which can persist in subsequent mitoses. This results in unequal chromosome numbers in the embryo-sac nuclei (table 5). Bridges persist through interphase because no cell walls are formed. Restitution at the next division results in polyploid nuclei.

Micronuclei can be formed by fragments from inversion crossing-over as well as by lagging univalents. One unusual embryo-sac of *Dalhansoni* had two " near diploid " early anaphases. Each of these had 23 chromosomes and one had in addition the products of a delayed but active micronucleus (plate 2, fig. 8). The two " near diploid " nuclei, instead of one haploid and one triploid, result from abnormal differentiation. The micronucleus was probably formed by a lagging univalent. At M III the chromosomes in the micronucleus

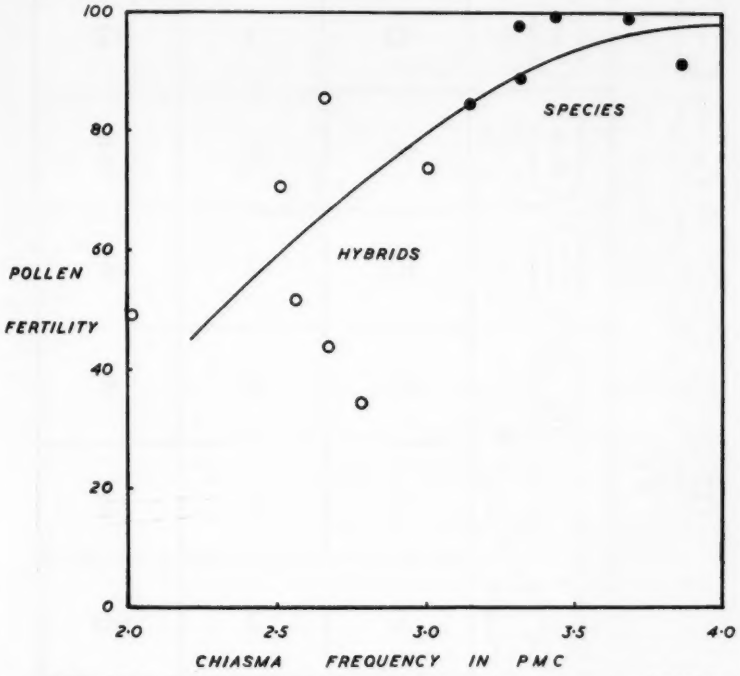

FIG. 3.—Relation between pollen fertility and chiasma frequency per potential bivalent in PMC of *Lilium* species and hybrids.

became active but delayed behind the main nucleus. The centromeres have separated while the chromosomes remain not fully spiralised.

Errors of synchronisation and differentiation.—The formation of the secondary four-nucleate embryo-sac with two haploid and two triploid nuclei depends on migration of one nucleus from the micropylar to the chalazal end and on synchronisation of mitoses in the three basal nuclei. The development of this stage is frequently abnormal in hybrids. Two tetraploid ; four diploid ; or four haploid plus two diploid nuclei being formed instead of two haploid and two triploid.

Failure of synchronisation occurs at the second division of meiosis in PMC of hybrids (table 3) but because of wall formation this has

no consequence. A similar error in PMC of *Tradescantia* (Haque, 1953) is accompanied by failure of wall formation at second division

TABLE 6

Abnormal embryo-sac development in Lilium *hybrids. Individual figures for Edna Kean, Lilian Cummings, Lyla McCann and Phyllis Cox have been totalled and shown as Stenographers*

NORMAL	ABNORMAL	DALHANSONI	MARHAN	STENOGRAPHERS
x	2x	0	1	12
x x	2x 4x or 2x 4x	4	5	7
x x x x	x 2x x	10	1	14
x 3x	x 3x Degen.	11	0	3
x 3x	MICRONUCLEI	2	2	13
		2	3	68
		4	1	15
TOTAL ABNORMAL		33	13	132
TOTAL NORMAL		57	44	285

of meiosis. This results in binucleate pollen grains in some of which mitoses are not synchronised. A similar error in the embryo-sac would prevent the fusion of the three basal nuclei and produce abnormal chromosome distribution.

Similar errors in chromosome distribution in the embryo-sac can arise from errors in cytoplasmic differentiation which controls the migration of the nucleus from the micropylar to the chalazal end of

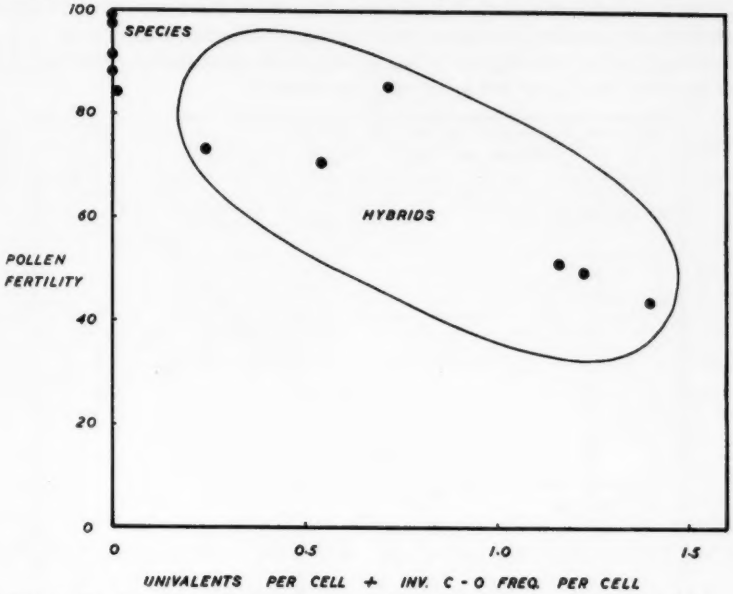

FIG. 4.—Graph showing relation of pollen fertility with the mean number of univalents per PMC and inversion crossing-over frequency per PMC in *Lilium* species and hybrids.

the embryo-sac. La Cour (1949) has shown the importance of cytoplasmic differentiation in the development of the pollen grain. The maternally determined cytoplasmic gradient in the embryo-sac can

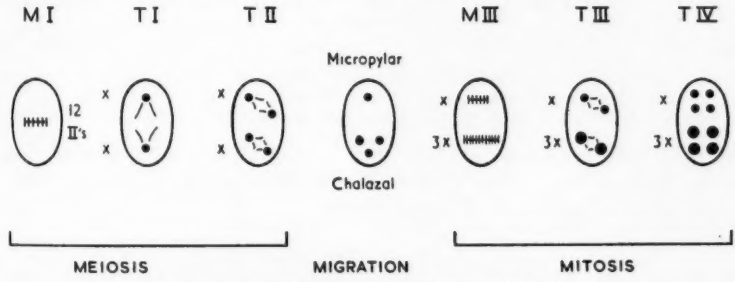

FIG. 5.—Normal embryo-sac development in *Lilium*
x = 12. II = bivalent.

presumably be influenced by the products of segregation, the haploid nuclei. In these hybrids the result of such interactions are observed as abnormalities in chromosome distribution.

A further abnormality occurred at M IV in some of the species and hybrids where the basal triploid nucleus fails to divide, and sometimes degenerates. This results in seven or six nuclei in the mature embryo-sac but it probably has little effect on embryo-sac fertility for it merely reduces the number of the antipodal nuclei and does not affect the egg or polar nuclei. In calculating embryo-sac normality these have been regarded as normal.

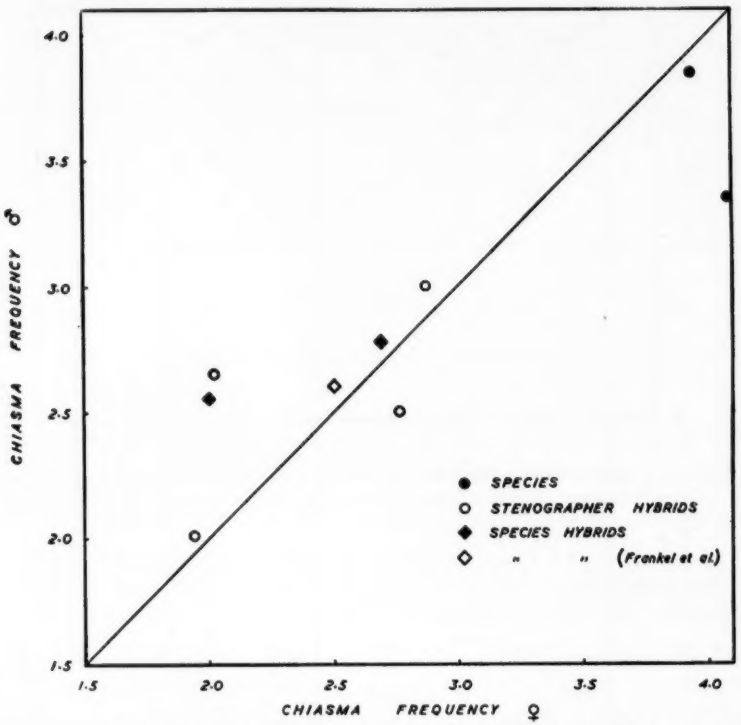

FIG. 6.—Chiasma frequency per potential bivalent of ♂ and ♀ cells plotted against one another. Deviations from equality not significant.

The level of embryo-sac sterility is similar to pollen sterility in these hybrids but it has a more direct effect on the fertility of the plant as measured by seed development.

5. SEED DEVELOPING AFTER SELFING

Based on seed development the species are either self-fertile or self-sterile (table 7). This behaviour suggests an incompatibility system such as pollen-style incompatibility but examination of pollen tube growth after selfing in fertile and sterile forms fails to support this suggestion. In *L. regale*, which is self-fertile, pollen tubes had reached

the base of the style (60 mm.) three days after pollination and were at the ovule five days after pollination. In *L. pardalinum*, which is self sterile, pollen tubes were normal in appearance and had reached the base of the style five days after pollination. Fertilisation could not be definitely established and there were no ovules developing three weeks after pollination. Thus the incompatibility reaction is

TABLE 7

Pollen fertility, embryo-sac normality and seed development three weeks after self pollination, in Lilium *species and hybrids*

	Pollen fertility	Embryo-sac normality		Seed development after selfing			
	Per cent. good pollen	No. of embryo-sacs	Per cent. normal	No. of ovaries	Ovary swelling ovule devel.	No. ovules examined	Per cent. with devel. endo-sperms
SPECIES—*Self sterile*							
L. candidum	84·5	14	100	2	+	312	0
L. Davidi	97·4	...	...	3	−	...	...
L. Hansonii	88·1	20	100	3	−	...	...
L. Henryi	98·9	...	...	2	−	...	...
L. pardalinum	98·3	114	100	12	−	...	...
SPECIES—*Self fertile*							
L. regale	91·3	152	99·4	12	+	115	95·6*
L. Szovitsianum	96·7	...	...	2	+	468	3·0
F_1's							
Marhan	51·5	62	72·6	3	+	150	14·7*
Dalhansoni	34·5	102	74·5	4	+	624	1·1*
MULTIPLE HYBRIDS							
Lyla McCann	49·3	44	38·6	5	+	684	1·5*
Lilian Cummings	70·6	137	68·6	5	+	1050	5·4
Edna Kean	85·5	210	73·3	3	slight	762	0
Phyllis Cox	73·1	65	70·8	6	+	1482	4·6*
Coronation	43·8	...	...	5	slight	1080	0

Note.—Pollen fertility is based on examination of 800 to 1200 pollen grains for each species and hybrid.

* Including some endosperms with spontaneous chromosome breakage.

determined very late and it may occur after fertilisation as has been suggested for *Hemerocallis* (Stout and Chandler, 1933), *Gasteria* (Sears, 1937) and *Theobroma* (Knight and Rogers, 1953).

Spontaneous chromosome breakage, resulting in degeneration of the endosperm and abortion of the embryo has already been reported in *Lilium*. This occurs frequently in hybrids and occasionally in the self fertile species, *L. regale*, and it may constitute the mechanism of incompatibility in the self sterile species. The swelling of the ovaries of *L. candidum* and *L. Szovitsianum* suggests a response either to pollination or to fertilisation and supports the view that seed development may fail after fertilisation. But it was not possible to establish that fertilisation had occurred.

In the hybrids, however, every ovary swelled and three weeks after pollination there had been considerable failure of ovules after fertilisation.

Although the full explanation of the seed failure in the species cannot be given it is clear that, in the hybrids, the failure of seed development after fertilisation is more important in determining fertility than either the pollen or the embryo-sac fertility.

6. SUMMARY

1. Fertility in *Lilium* hybrids, measured by seed development after selfing, is reduced by pollen sterility, embryo-sac sterility and failure after fertilisation.

2. Some are structural hybrids with reduced chiasma frequency, univalents at M I, and crossing-over in inverted segments.

3. Segregation is responsible for differences in nuclei. These have different mitotic rates and cause errors in cytoplasmic differentiation in the embryo-sac.

4. Spontaneous chromosome breakage in the endosperm of hybrids is followed by degeneration of the fertilised ovule.

5. Sterility in these hybrids is thus seen to arise from errors at three stages :

(i) Meiosis—Reduced chiasma frequency and inversion crossing-over.

(ii) Mitosis—Errors of differentiation and synchronisation.

(iii) After fertilisation—Endosperm failure.

6. In self-sterile species pollen tube growth is normal and the sterility mechanism seems to operate at or after fertilisation.

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Plate 1

Normal embryo-sac development in *Lilium*

Fixation Carnoy fluid (6 : 3 : 1). Stained Feulgen reagent. Dissected end squashed in 45 per cent. acetic acid.

FIG. 1.—Pachytene.

FIG. 2.—First metaphase of meiosis (M I) 12 bivalents.

FIG. 3.—Second metaphase of meiosis (M II). Chromosomes 12 : 12.

FIG. 4.—Second anaphase of meiosis (A II). Chromosomes 12 : 12 : 12 : 12.

FIG. 5.—Interphase after T II, migration of one nucleus from micropylar to chalazal end of embryo-sac.

FIG. 6.—Anaphase (A III) producing secondary four nucleate embryo-sac. Chromosome distribution 12 : 12 : 36 : 36.

All figures : ×380.

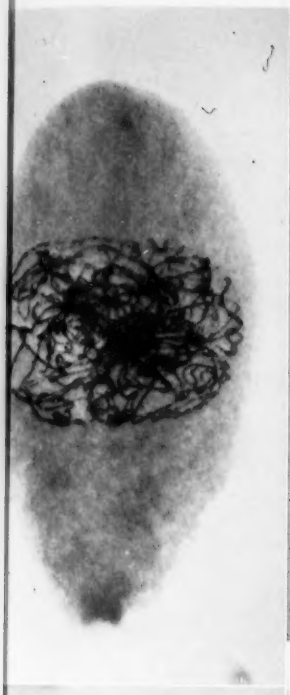

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Plate 2

Abnormalities in embryo-sac development in *Lilium* hybrids. Technique—as for plate 1

FIG. 1.—Inversion crossing-over bridge and fragment at A I. Bridge being carried complete to one nucleus. Chromosome distribution 11 : 11+dicentric. ×

FIG. 2.—Interphase bridge after first division of meiosis. Restitution at next division will result in diploid nuclei.

FIG. 3.—Inversion crossing-over bridge and fragment at A II. Chromosome distribution 13 : 13+C2 bridge, 10 : 10.

FIG. 4.—Fusion of spindles of chalazal nuclei at M III. Also a micronucleus from abnormality at M I and an acentric fragment from abnormality at M II. Chromosome distribution probably 12 : 36.

FIG. 5.—Failure of spindle fusion at M III resulting in *x*, *x*, 2*x*, 2*x*, *x*, *x* instead of *x*, *x*, 3*x*, 3*x*.

FIG. 6.—M III showing dicentric chromosome in 3*x* nucleus. This results from inversion crossing-over bridge at A II being carried complete. Chromosome distribution 12 : 34+dicentric.

FIG. 7.—T II with micronucleus.

FIG. 8.—One of the two very early anaphases at A III showing products of delayed but active micronucleus. Chromosome distribution 23 : 23+active micronucleus.

Figs. 1-7 : ×380. Fig. 8 : ×1450.

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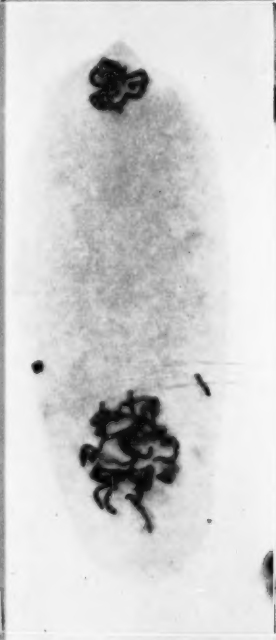

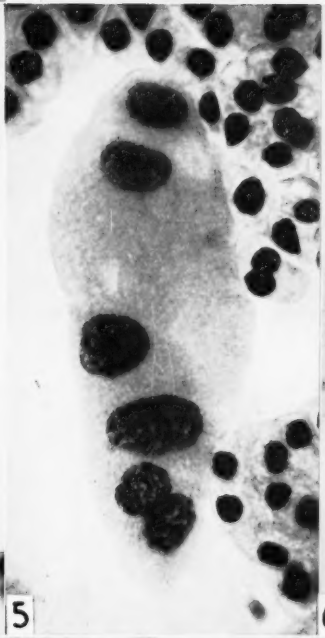

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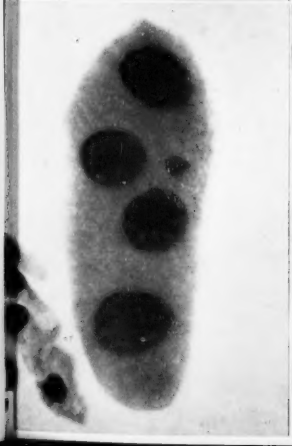

8

HORMONE-INDUCED PEAR-APPLE HYBRIDS

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1. INTRODUCTION

MANY intergeneric hybrids are known, and in the Pomoideæ attempts to produce such hybrids by plant breeders have met with some success. A sexual hybrid between pear and apple was produced by Crane and Marks (1952) by using a growth regulating substance or hormone, β-naphthoxyacetic acid, on the pear ovary at the same time that it was pollinated with apple pollen.

Hormones have of recent years been used for two purposes. First there is the production of parthenocarpic fruit without pollination (Avery and Johnson, 1947; Zimmerman and Hitchcock, 1948). Osborne and Wain (1951) obtained parthenocarpic apples and pears in this way. Of the substances they tested only one, α-(2-naphthoxy) propionic acid, was effective in producing mature fruit on pears and then only on a limited number of varieties. This substance was ineffective when applied to apples. A different substance, however, α phenoxypropionic acid, produced mature apples but again on only one of the three varieties tested. Thus the induction of parthenocarpic fruits in *Pyrus* seems to be a highly specific reaction.

Secondly, hormones have been used as an aid to crossing. Emsweller and Stuart (1948) found that for giving hybrids between species of *Lilium*, the most effective was naphthalene acetamide.

It is likely that the action of the hormones in assisting hybridisation is related to parthenocarpy. These studies were aimed at finding out the mode of action of β-naphthoxyacetic acid and also the reason for its rather limited effectiveness as an aid to crossing pears and apples.

2. MATERIALS AND METHODS

Crosses were attempted between various varieties of pears and apples. Immediately after pollination the ovary and base of the styles were brushed once with a 40 parts per million (p.p.m.) aqueous solution of β-naphthoxyacetic acid, care being taken to ensure that the hormone solution did not come in contact with the stigma or the pollen. Nine varieties of pears were used as female parents. Seven were diploid, one was triploid and one, Fertility Improved, was a periclinal polyploid chimera with diploid epidermis and tetraploid inner tissue (Marks, 1953).

Each pear variety was selfed and crossed with a diploid apple variety, Cox's Orange Pippin and with a tetraploid apple variety, 16-36 AT. In addition self and cross pollinations without hormone treatment were made on Fertility. All of the pear varieties were self incompatible except the tetraploid Fertility Improved, so compatible pear-pear pollinations were made between Fertility and Conference. Conference flowers were also pollinated with cherry and plum pollen.

Three diploid apple varieties were used as female parents. These were selfed and crossed with Fertility (2*x*) and Fertility Improved (4*x*) pears.

The fruit and seed set were recorded and the development of the embryo-sac, fertilisation and the early development of the embryo and endosperm were followed. The effect of hormone on rate of pollen tube growth was studied both *in vitro* and *in vivo*.

For embryological studies buds were fixed in Navashin fluid at daily intervals from meiosis until fourteen days after pollination. After embedding in wax, sections were cut 12 μ, 16 μ or 20 μ thick and stained in crystal violet. Pollen tube growth of Cox apple and Fertility pear was measured after germination on cellophane floated on hormone in 15 per cent. sucrose (Darlington and La Cour, 1947). Pollen tube growth in pear styles, 1, 2, 4 and 8 days after pollination, was measured after fixation in acetic alcohol (1 : 3) and storage in 30 per cent. alcohol. Before examination they were softened in water for a few minutes, cut longitudinally, stained in 0·4 per cent. cotton blue in lacto-phenol and mounted in water, cut surface uppermost.

3. FRUIT AND SEED SET

Table 1 summarises the results of the crossing experiments and the following points are apparent :

(i) Parthenocarpy was induced in four of the pear varieties but in none of the apples.

(ii) Parthenocarpy requires hormone but does not require pollination.

(iii) Pear-apple hybrids were only formed in varieties capable of parthenocarpy.

(iv) The naturally parthenocarpic variety, Fertility, was the most favourable for hybridisation.

(v) Hormone-induced parthenocarpy and very slight seed formation occurred in self incompatible varieties. This seed setting may be due to the breakdown of the incompatibility system when the flowers are kept on the plant for longer than normal (East, 1923), or to parthenocarpy allowing recovery of single-seeded fruits which would have otherwise been lost (Lewis, 1948).

4. EMBRYO-SAC DEVELOPMENT

In all the varieties of pears the embryo-sac developed from an anatropous ovule with a thick nucellus. This differed slightly from the behaviour in apples described by Wanscher (1939) where a hypodermal cell of the nucellus developed into the megaspore mother cell. Meiosis occurred approximately six days before anthesis and resulted in a T-shaped tetrad. The embryo-sac development was monosporic, the chalazal megaspore divided to give a two-celled embryo-sac four days before anthesis, a four-celled embryo-sac three days before anthesis, and an eight-celled embryo-sac one to two days before anthesis. At or shortly after anthesis the embryo-sac was organised into egg, synergids, polar nuclei and antipodals.

Fusion of the polar nuclei occurred between two and eight days after anthesis. The number of antipodal cells also varied. The

maximum number observed was eighteen in one embryo-sac of the variety Conference. The modal number of antipodals varied from five to eight in the different varieties.

TABLE 1

Results of crossing experiments with pears and apples using hormone treatment

♀ Parent	♂ Parent	Flowers pollinated	Mature fruit	Total seeds	Seeds per fruit	Per cent. germination
A. Successful parthenocarpy or hybridisation						
Fertility (2x)	Compatible Pear	30	7	53	7·6	*98·1*
	2x Apple	105	25	155	6·2	*82·6*
	4x Apple	105	32	157	4·9	*82·2*
	Selfed	35	15	1	0·07	*(100·0)*
	No pollen	30	12	0	...	...
Conference (2x)	2x Apple	30	3	0	...	...
	4x Apple	30	4	3	0·75	*33·3*
	Selfed	80	14	3	0·22	*66·7*
	Cherry	150	6	0	...	...
	Plum	150	10	0	...	...
Doyenné du Comice (2x)	2x Apple	30	2	0	...	...
	4x Apple	30	3	9	3·0	*100*
	Selfed	30	0	...	...	...
Fertility Improved (4x)	2x Apple	30	1	0	...	...
	4x Apple	30	0	...	...	...
	Selfed	30	12	74	6·2	...
B. Unsuccessful parthenocarpy and hybridisation						
PEARS						
Clapp's Favourite (2x)						
Dr Jules Guyot (2x) .	2x Apple	30	0			
Louise Bonne de Jersey (2x)	4x Apple	30	0			
Seckle (2x) . .	Selfed	30	0			
Beurré d'Amanlis (3x)						
APPLES						
Cox (2x) . . .	2x Pear	30	0			
*Lord Derby (2x) .	4x Pear	30	0			
Merton Prolific (2x) .	Selfed	30	0			

* One selfed fruit bearing one seed was obtained from Lord Derby.

Note.—Twenty-five flowers of Fertility (2x) with 2x apple, 2x pear, and no pollen gave no mature fruit.

Unfertilised ovules showed no sign of degeneration up to fourteen days after anthesis. There were no consistent differences in the form or timing of the embryo-sac development and it is therefore unlikely that variation in embryo-sac development is responsible for the difference in seed setting after hybridisation.

5. POLLEN TUBE GROWTH AND FERTILISATION

Smith (1942) and Addicott (1943) both obtained stimulation of pollen germination and pollen tube growth by optimal concentrations of hormones and vitamins. Indolacetic acid stimulated at a concentration of 10 p.p.m. High concentrations retarded both growth and germination. Addicott suggested that germination and growth are stimulated independently.

In the experiments with apple and pear pollen grown on cellophane the tube growth was stimulated at concentrations of β-naphthoxy acetic acid between 1 p.p.m. and 10 p.p.m. (fig. 1). It is notable

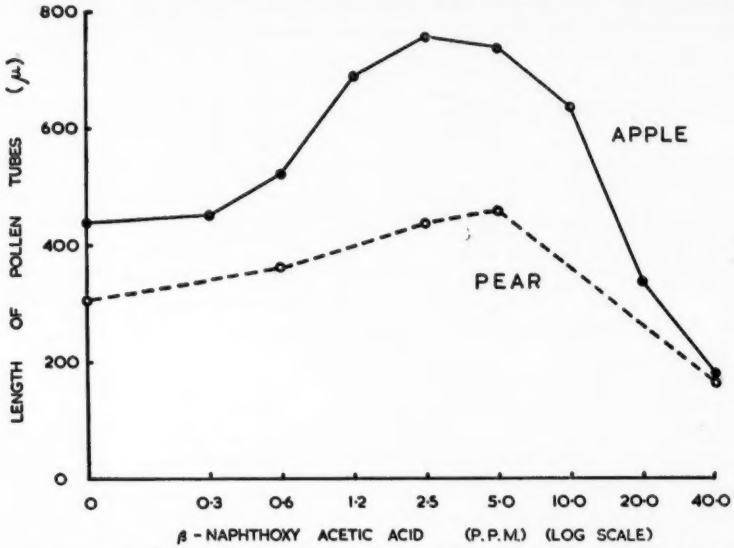

FIG. 1.—Effect of various concentrations of β-naphthoxy acetic acid on amount of tube growth of Cox apple and Fertility pear pollen. Pollen germinated on cellophane on 15 per cent. sucrose and hormone. Mounted in 0·08 per cent. cotton blue in lacto phenol after growth for 15 hours at *ca.* 18° C. and constant humidity.

that in crosses the hormone is applied to the base of the style at a concentration of 40 p.p.m. At this concentration the pollen germination is inhibited (table 2) and the tube growth is slow and abnormal; many tubes had swollen ends or had burst.

Although the pear pollen tubes grew more slowly than the apple a similar stimulation was induced at the same optimal concentrations.

When growing in pear styles, apple pollen grew more slowly than pear pollen. The application of hormone stimulated the growth of the apple pollen (fig. 2), but it did not seem to affect the growth rate of the pear pollen. This was in general agreement with the results of Modlibowska (1945).

No differences were observed in the growth rate of pollen from

diploid apple and that from the tetraploid when they were growing in pear styles. In all cases the growth of the apple pollen tubes, although slow, appeared normal.

Fertilisation occurred from five to seven days after pollination in the compatible pear-pear crosses. In the successful pear-apple crosses the pollen tubes did not reach the embryo-sac until ten to twelve days after pollination. Where no hormone was applied no pollen tubes had reached the embryo-sac fourteen days after pollination (fig. 2).

Fertilisation studies on the variety Beurré d'Amanlis, which failed to set any seeds, indicated that after hormone treatment the apple pollen tubes reached the embryo-sac at approximately the same time as in Fertility, ten to twelve days after pollination, thus indicating

TABLE 2

Effect of various concentrations of β-naphthoxyacetic acid on germination of Cox apple and Fertility pear pollen (cf. *fig. 1*)

Concentration of β-naphthoxy acetic acid	Apple		Pear	
	Total pollen grains	Per cent. germination	Total pollen grains	Per cent. germination
0	597	*78·4*	546	*84·6*
2·5 p.p.m.	604	*83·8*	572	*89·7*
5·0 p.p.m.	606	*81·8*	602	*79·7*
40 p.p.m.	606	*42·7*	674	*47·6*

that the reason for the failure of hybridisation, in Beurré d'Amanlis at least, was not failure of fertilisation. Although Beurré d'Amanlis is a triploid, pollen from compatible diploid pears gives good fruit set and some seed (Crane and Lewis, 1942), so here the hormone, although assisting fertilisation, failed to prevent abscission and the young hybrids were lost.

In the pear-cherry and pear-plum pollinations no pollen tubes had reached the embryo-sac by fourteen days after pollination.

6. CHARACTERS OF THE HYBRIDS

Chromosome number.—Proof of the hybrid nature of the seed was given by cytological examination of the seedlings. Six seedlings from the cross diploid pear × tetraploid apple were all found to be triploid ($2n = 51$). Six seedlings from the cross diploid pear × diploid apple were all diploid ($2n = 34$).

Seed germination and seedling growth.—Both the diploid and the triploid seed from the pear-apple crosses had 82 per cent. germination as compared with 98 per cent. germination for the seed from the pear-pear cross. As with the seedlings grown by Crane and Marks (1952), the subsequent development was, however, unusual. Within a month some of the seedlings were wilting and lacking in vigour. The triploid seedlings were the most affected and after four months 85 per

cent. of the triploid seedlings and 39 per cent. of the diploid seedlings had died.

The seedlings appeared to have a defective root system and grafts were made to apple and pear stocks by Mr A. G. Brown. Preliminary

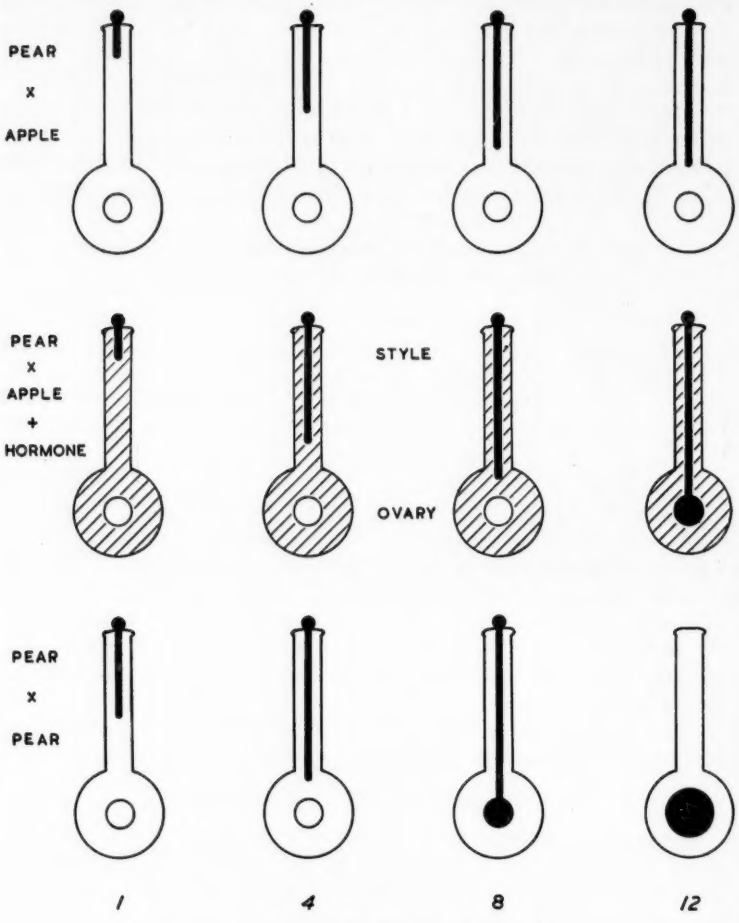

FIG. 2.—Diagram of pollen tube growth and fertilisation in pear × pear and pear × apple crosses.

results suggest that when grafted they grow much better. More success has been achieved with apple stocks but some grafts are growing well on pear stocks.

External characters.—As the hybrid seedlings were only observed during their first season it was not possible to define their genetic

characters with certainty. The hybrids developed intermediate characters and were distinct from either apple or pear seedlings. Crane and Lewis (1949) have listed various genetic characters for pear varieties and Lewis and Crane (1938) some for apple varieties. Table 3 is an attempted classification of the F_1 pear-apple hybrids using these characters. The hairiness of the summer shoots and the serrate leaf margins resembled the apple parents and the dull green leaf colour was like that of apple seedlings. In the cross involving Comice, however, the pale red colour of the stem and the relatively

TABLE 3

Characters of pear-apple hybrids and parent varieties

Variety or hybrid	Summer shoots	Leaf margins	Glands on main leaf midrib	Leaf surface
PEAR				
Fertility (2*x*)	Red, sub-glabrous	Serrate-crenate	Red	Shiny
Comice (2*x*)	Pale red, sub-glabrous	Crenate	Few, red	,,
APPLE				
Cox (2*x*)	Red, hairy	Serrate	Green	Dull
16-36 AT (4*x*)	Red, hairy	Serrate	Green	Dull
PEAR-APPLE F_1				
Fertility × Cox (2*x*)	Red, hairy	Serrate	Red	Dull
Fertility × 16-36 AT (3*x*)	Red, hairy	Serrate	Red	Dull
Comice × 16-36 AT (3*x*)	Pale red, hairy	,,	Few, red	,,

few glands on the leaf midrib resembled the pear parent. Many of the hybrid seedlings had occasional lobed leaves, a character which is commonly observed in pear seedlings, but rarely in apple seedlings.

7. DISCUSSION

In considering the reasons for the success of the hormone in assisting fertilisation two effects have been demonstrated ; it stimulates pollen tube growth and induces parthenocarpic fruit set.

With hormone, pear-apple fertilisation is delayed by comparison with pear-pear fertilisation and without hormone it does not occur within fourteen days, if at all. The importance of this delayed fertilisation can be best assessed by a consideration of the mechanism of fruit drop in the pome fruits.

Luckwill (1952) emphasises the relationship between development of the endosperm, the auxin content of the developing seed, and the time of fruit drop in apples. The first two to three weeks after petal fall are characterised by the virtual absence of auxin in the seed. During this period the endosperm is non-cellular and developing

rapidly. It becomes cellular after about three weeks and at the same time auxin first appears in the seed. This coincides with the end of the "first drop". Once the endosperm becomes cellular it begins to produce a secondary endosperm and in doing so much of the primary endosperm is digested. Thus there is an initial decrease in the amount of endosperm followed by a rapid rise. This period of decrease is correlated with a low auxin content and coincides with the period of the "June drop".

Thus we can see that the fate of a late fertilised hybrid depends very largely on the auxin content of the ovule, which in turn is related to its stage of growth. It is very unlikely that the late fertilised pear-apple hybrid will be capable of producing natural auxins in time to prevent abscission. In naturally parthenocarpic varieties, however, the supply of auxin is less dependent on the development of the seed and can be supplemented by the application of hormone. Hence the success of the technique employed here is dependent, first on the stimulation of the pollen tube growth which allows fertilisation to take place, and secondly on a supply of hormone which prevents the formation of the abscission layer.

The reason for the failure of the pear-apple cross with most varieties and the complete failure of the reciprocal apple-pear cross is probably the inability of these varieties to react to the hormone in preventing the formation of the abscission layer, which in turn is related to their genetically lower auxin production. This suggests that in naturally parthenocarpic fruit an alternative site of hormone production is more strongly developed. Incidentally, intergeneric hybrids in the Pomoideæ have been most easily made in the past with varieties which are naturally parthenocarpic.

Osborne and Wain (1951) have shown that parthenocarpic fruit set is possible on apples. But a different hormone from the one successful on pears is necessary. Failure of fertilisation may contribute to the lack of success in apples. The slower growing pear pollen tubes (fig. 1) have a longer path to travel (apple styles 10 mm., pear styles 6 mm.) and the apple styles degenerate six to eight days after they become receptive.

Dr W. O. James tells me that the growth of roots by barley seedlings on White's culture solution is much improved by the addition of an extract from the endosperm. Thus the endosperm seems to produce a substance which is necessary for normal root growth and in this way the later beginning of the hybrid seeds is probably related to the poor root growth of the young seedlings.

The effect of the hormone in inducing parthenocarpy is much more specific than its effect on stimulation of pollen tube growth. Hence further hybridisation should be based on the selection of varieties and hormone treatments which, through parthenocarpic fruit development, will provide a medium for the late developing hybrid seeds and prevent loss through abscission.

8. SUMMARY

1. Hybridisation and parthenocarpy were produced by applying β-naphthoxyacetic acid to the base of the style of the pear at the same time as the apple pollen was applied to the stigma.

2. The hormone stimulated the growth of the apple pollen tubes and thus allowed fertilisation to occur, but this was later than pear-pear fertilisation.

3. The success of the cross also depends on the effect of the hormone in inducing parthenocarpy and preventing the loss of the late developing hybrids.

4. The growth of the pear-apple seedlings was abnormal but four months after germination 37 per cent. still survived. The seedlings were otherwise intermediate in character between pears and apples.

Acknowledgment.—This work was done while at the John Innes Horticultural Institution, Bayfordbury, Herts. on a Studentship from the Commonwealth Scientific and Industrial Research Organisation, Australia. I wish to express my thanks to Dr C. D. Darlington and Mr M. B. Crane for facilities, helpful advice and criticism.

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REVIEW

NEW CONCEPTS IN FLOWERING-PLANT TAXONOMY. By J. Heslop-Harrison. London : Heinemann. 1953. Pp. 135. 5s.

The beginnings of genetic ecology by Turesson and of chromosome systematics by Tischler are now 30 years old. Since these beginnings new ideas and new methods have been growing up over the whole field of plant and animal classification. They have shown that the static assumptions of Linnaeus and of the modern museum are in conflict with the evolutionary assumptions made by genetics. In the nineteenth century this conflict was concealed in abstractions. It is now open and obvious.

Dr Heslop-Harrison in this little book dissects and describes this conflict. He does so without timidity and without evasion. His table (pp. 198-99) showing the opposition of classical and experimental systematics is a proper climax to his argument. It stands also in pointed antithesis to the opinion given by Dr W. B. Turrill in his Foreword that the practice of plant classification has been " adjusted " to the coming of the theory of evolution. Of course it has not been adjusted ; it is not being adjusted ; and its practitioners are well organised to resist the danger of having to adjust it. It is to its meeting this resistance that Dr Harrison's book owes its chief value.

Dr Harrison's sympathies are, no doubt, in favour of the experimenters and evolutionists : otherwise he could not be so unkind to the namers and describers. They, however, should know that he has not been too kind to the evolutionists. He has dispensed with the modern evidence of cultivated plants. Yet that evidence has done as much for the understanding of natural variation in our hands as it did in Darwin's hands. He has also denied himself the use of the facts and ideas derived from chromosome studies during the last 25 years. *Erophila* appears but without the polyploidy of Winge ; *Taraxacum* appears but without the mutant chromosome types of Sørensen and Gudjonsson. He has failed to note the development of the new sciences of chromosome ecology and chromosome geography. The works of Manton, Löve, Barber, Fernandes, Janaki Ammal, Stebbins, Stern and Skalinska, in this field pass unrecorded.

Can it be that Dr Harrison is tempering the cold wind of experiment to the shorn lamb of systematics ? It seems not. It seems rather that he has been content to study the new aspects of his subject at second hand. In his arrangement of the book he puts systematics first, breeding second, and the chromosomes last. Can it be that he looks at his problems in this order and sees the chromosomes as the end of the story of variation and not as the beginning ? Probably. For on this view many things are explained. It is natural that he should say that " without a reasonably full fossil record phylogeny cannot be positively determined ". He merely means that the body of inference from the chromosomes is negative or secondary or fallacious. He has not noticed that they can preserve a record of phylogeny of its own kind more certain than any fossil record, a principle which has been known for twenty years or more.

It is also natural that Dr Harrison should ascribe mutation in apomictic

strains of plants to a mysterious process of " autosegregation " which should " theoretically " not occur. If he had meant that the name should not occur we might have agreed. But the thing and the theory, the crossing over without effective meiosis, the system of subsexual reproduction, these have been known for twenty years or more.

Again it is natural that Dr Harrison should forget that inbreeding does not always produce a homozygote : it sometimes produces *Œnothera*. In producing *Œnothera* it has produced the most thoroughly investigated of all problems in the formation of plant species. Which we have likewise known for twenty years or more.

Both in the text and in his glossary Dr Harrison defines his terms. In these definitions we encounter even deeper difficulties. To him adaptation (like mutation to some geneticists) is not a process so much as a " character " (p. 123). Now what he means by a character is not clear. Sometimes he may mean a genetically determined difference ; but sometimes (p. 48) he certainly means a physiologically determined difference. A biotype, on the other hand, is a group of " individuals which are genotypically all essentially the same " (p. 123). This would be right enough if we were told what was essential. But when he come to individuals themselves he changes his system. He does not define them by genotype at all. They are merely plants with " physiological independence " (p. 13). Different potato plants of a clone are therefore different individuals and potato " strains ", by which he means clonal varieties, " exist in populations of thousands or millions of individuals " (p. 31).

Does Dr Harrison misunderstand the foundations of the subject ? Or is he merely confused in presenting them ? We cannot be sure. But we may venture an opinion. It is that if Dr Harrison had cleared his book of the biotypes and karyotypes, topoclines and cytodemes, autosegregations and agamospermies and if he had left a space for the fundamental notions of what constituted an individual, a hybrid, and a strain, he would have served his purpose better.

We must, nevertheless, be grateful to Dr Harrison. What he writes, although most of it might have been written some years ago, in fact was not written by any systematist at that time. He has gone half way to meet genetics. It is only unfortunate that half way is not enough.

C. D. Darlington.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND FIFTEENTH MEETING of the Society, held on 21st and 22nd SEPTEMBER 1954, at KING'S COLLEGE, Newcastle upon Tyne

MAPS OF A CHROMOSOME REGION IN *ASPERGILLUS NIDULANS* BASED ON MITOTIC AND MEIOTIC CROSSING OVER

G. PONTECORVO and E. KAFER
Dept. of Genetics, University of Glasgow

The *bi* chromosome of *A. nidulans* has been thoroughly mapped by means of the standard techniques developed for homothallic fungi. This has permitted a comparison with the maps based on mitotic recombination. The orders of the genes, of course, agree, including the position of the centromere as identified by tetrad analysis by Mr Strickland. The relative distances have been calculated for three regions (centromere—*pro* 1—*paba* 1—*y*) and they show an excess of mitotic crossing-over in the region *pro*—*paba*. The difference is not, however, very large.

This first example of a chromosome map based on mitotic crossing-over shows that formal genetic analysis can be carried out perfectly well outside the sexual cycle. It is conceivable that a good deal of the formal genetics of slow-breeding higher animals, including man, and plants could now be done on tissue cultures.

THE RELATIONSHIP BETWEEN A GROUP OF ALLELES IN THE "ad" REGION OF *ASPERGILLUS NIDULANS*

R. H. PRITCHARD
Dept. of Genetics, University of Glasgow

Nine allelic mutants requiring adenine for growth, and almost certainly of independent origin, have been used in a study of crossing-over between alleles. The mutants are indistinguishable in qualitative tests for response to alternative growth factors, but two differ from the rest in showing limited growth on minimal medium.

In every combination so far tested, crosses between pairs of alleles have yielded adenine independent types. The majority of these certainly result from crossing-over as shown by the exchange of markers, one 0·2 cMo. on one side and the other 6 cMo. on the other. If the remainder, carrying the parental combinations of markers, were due to back mutation, then the mutation rate would be very high (10^{-4} to 10^{-5}), and (*i*) it would be at least 1000x the mutation rate observed in conidia, and (*ii*) it would be dependent on the particular combination of alleles tested. These apparent back mutants are more easily interpreted as multiple crossovers within a very short chromosome segment indicating intense negative interference within this segment.

One pair of alleles already tested shows the well-known position effect in diploids (*adxady*/++ = normal ; *adx*+/+*ady* = mutant). This property has been used for the recovery of the complementary products of mitotic crossing-over and the results so far support the interpretation of multiple crossing over within a very short chromosome segment (*ca.* 0·2 cMo.).

ALLELES OR PSEUDO-ALLELES ?

G. PONTECORVO
Dept. of Genetics, University of Glasgow

Four out of four of the groups of independently-arisen mutations in *Aspergillus*, and one out of one in *Drosophila*, investigated by various workers in this Laboratory, show crossing-over between mutants which behave as alleles to one another. The well-known position effect (double heterozygote in *cis* less mutant than double heterozygote in *trans*) has been detected unquestionably in two out of the four cases in *Aspergillus* and the one in *Drosophila*, and it is almost certain to occur in the other two cases. The characters affected are four nutritional requirements in *Aspergillus* (biotin, adenine, para-aminobenzoic acid and proline) and eye pigment in *Drosophila*. Together with the findings of E. B. Lewis, Green, Stadler and Race in organisms ranging from *Drosophila* to man, these results confirm the tentative conclusion drawn a few years ago that allelism and pseudo-allelism are one and the same thing.

AN INDEX FOR USE IN QUANTITATIVE TAXONOMIC PROBLEMS

H. T. CLIFFORD
Botany Dept., Durham Colleges (University of Durham)

A method of constructing an index for use when comparing two or more groups is proposed. Pairs of measurements for each group are plotted graphically. If the scatter diagrams obtained are approximately parallel, the pairs of measurements can be combined as follows. Draw a new set of axes such that one of them is parallel to the trend of the scatter diagrams. The other axis is calibrated in an arbitrary scale and the index values are obtained for each point by reading off its value from the new scale.

ENVIRONMENTAL DETERMINATION OF THE SEX RATIO OF A PLANT-PARASITIC NEMATODE

C. ELLENBY
Zoology Dept., King's College, Newcastle (University of Durham)

No abstract available.

S GENE STRUCTURE REVEALED BY THE CYTOPLASM

L. K. CROWE
John Innes Horticultural Institution, Bayfordbury

There is a difference in the behaviour of hybrids from reciprocal crosses between the self-incompatible species *Oenothera pallida* ($S_{1\cdot2}$) and the self-compatible species *Oe. trichocalyx* (*Sf.f*). This is caused by the effect of the cytoplasm on the pollen.

S_1 and S_2 pollen, developed from hybrids carrying both the *Sf* allele and the cytoplasm from *Oe. trichocalyx*, has retarded pollen tube growth in *compatible* styles. S_1 pollen from $S_{1\cdot2}$ with *Oe. trichocalyx* cytoplasm is similarly retarded. The incompatibility reaction of the pollen is not affected.

In the *Oe. pallida* cytoplasm growth of S_1 and S_2 pollen is not retarded.

These facts will be interpreted by assuming a new gene in the *S* complex and that the place of action of this gene has been changed from the gametophyte to the sporophyte by the cytoplasm.

S GENE STRUCTURE—NEW EVIDENCE FROM TETRAPLOIDY AND MUTATION

D. LEWIS
John Innes Horticultural Institution, Bayfordbury

In diploid pollen grains of tetraploids, *S* alleles interact to give dominance or competition. Mutant *S* alleles produced by X-rays and spontaneously have lost their " pollen " specificity.

When these specificity deficient alleles are incorporated into diploid pollen grains of autotetraploids they show the full competition but not dominance effects of their unmutated predecessors. This is evidence that the " pollen " gene of the *S* complex is not entirely inactivated by the mutation, and supports the conclusions from the cytoplasmic effects described previously.

NUTRITIONAL REQUIREMENTS AND HYBRID VIGOUR IN *DROSOPHILA*

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Although hybrid vigour is a physiological phenomenon, there have been few attempts to examine what physiological mechanisms determine the superiority of the F_1 compared with the parents. Nor have any efforts been made to see if quantitative nutritional requirements are genetically determined. With the development of a satisfactory synthetic culture medium for *Drosophila melanogaster* both problems can be examined in a limited fashion. It will be shown that the minimal nutritional needs of pure lines differ, and that the minimal requirement of their F_1 is less than that of the parents for some dietary components, whereas for others it is like that of the " better " parent, or intermediate. Further, the hybrid shows capacities not found in either parent, and these apparently cannot be predicted from a knowledge of the characteristics of the parents. These findings will be discussed with respect to current theories concerning the mechanisms underlying hybrid vigour.

SOME GENETICAL FACTORS AFFECTING BODY SIZE IN *DROSOPHILA MELANOGASTER*

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A line has been selected for short wings until an apparently stable limit has been reached, about 20 per cent. smaller than the original wild type stock. At this point a number of inbred lines were formed which did not however differ in size. An isogenic line had also been formed, before the selection line reached its limit, and was about 8 per cent. larger than the inbred lines. It showed complete dominance when crossed to the inbred lines. A heterozygous 2nd chromosome of the isogenic line was substituted into an otherwise inbred genotype ; similarly for the 3rd chromosome. Combining selection with continued back-crossing to the recessive inbred parents, an attempt was made to bring to light any major factors on these chromosomes. The results are discussed.

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